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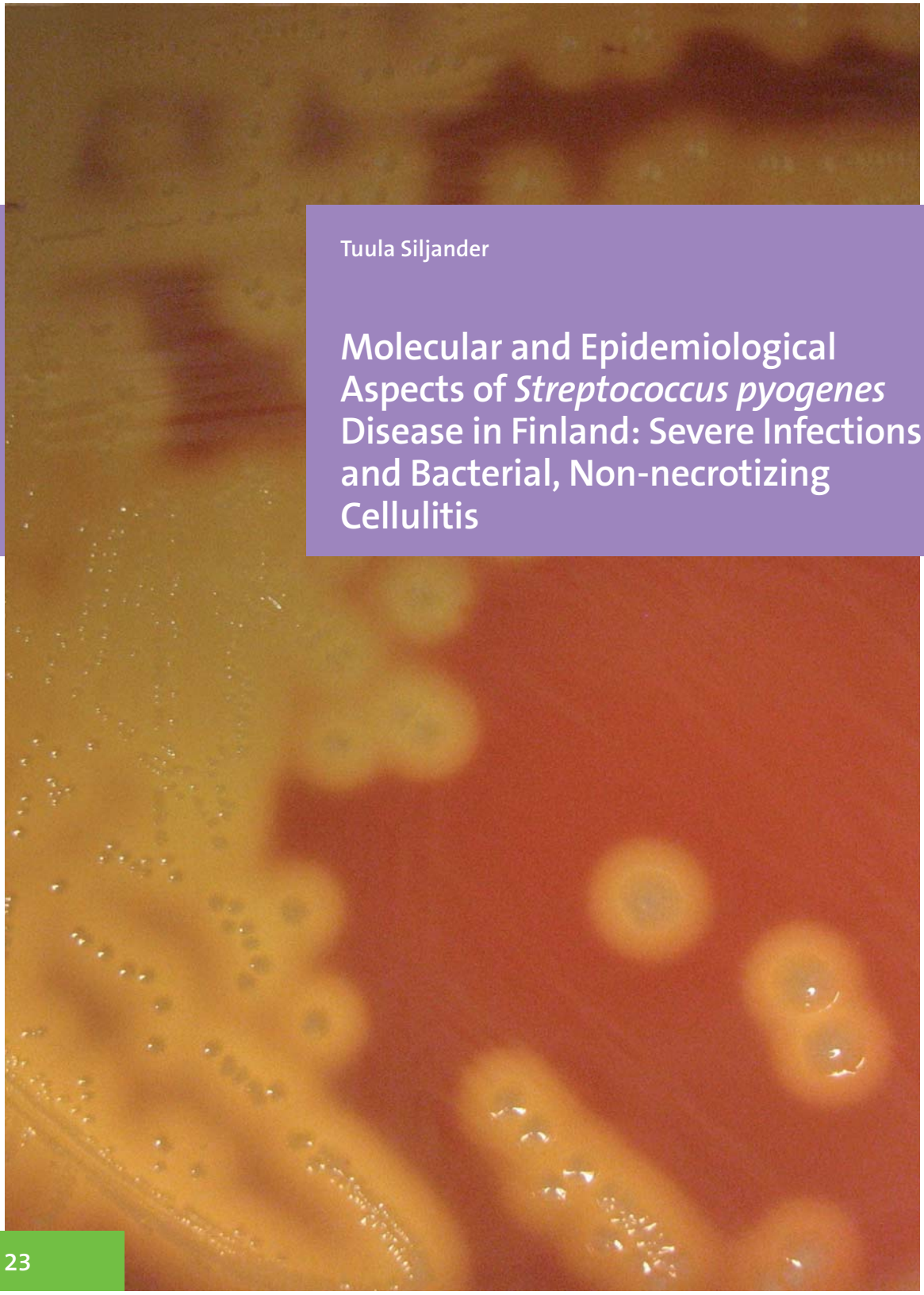

RESEARCH

Tuula Siljander

Molecular and Epidemiological Aspects of *Streptococcus pyogenes* Disease in Finland: Severe Infections and Bacterial, Non-necrotizing Cellulitis

Tuula Siljander

Molecular and Epidemiological Aspects of *Streptococcus pyogenes* Disease in Finland: Severe Infections and Bacterial, Non-necrotizing Cellulitis

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine, University of Helsinki, for public examination in the Small Lecture Hall, Haartman Institute, on December 4th, 2009 at 12 noon.

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Cover photo: Streptococcus pyogenes (type *emm*1) on a blood agar plate. The lysis of blood cells caused by the bacteria can be seen as a clear zone around the bacterial colonies.

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ABSTRACT

Tuula Siljander, Molecular and epidemiological aspects of *Streptococcus pyogenes* disease in Finland: Severe infections and bacterial, non-necrotizing cellulitis. National Institute for Health and Welfare, Research 23|2009. 160 pages. Helsinki, Finland 2009. ISBN 978-952-245-174-3 (print); ISBN 978-952-245-175-0 (pdf)

Background and aims. *Streptococcus pyogenes* (group A streptococcus, GAS) causes a variety of infections ranging from mild pharyngitis to severe, invasive infections such as bacteraemia. The predominant GAS strains in invasive disease vary over time and geographic region. In 2006, the Finnish nationwide surveillance showed an increase in invasive GAS disease, and clinicians were alarmed by the severe disease manifestations and poor outcome. These events prompted investigation of recent trends in incidence, outcome, and bacterial types. Bacterial, non-necrotizing cellulitis and erysipelas are localised and potentially recurrent infections of the skin. The aim of the study was to identify the β-haemolytic streptococci causing cellulitis and erysipelas infections in Finland.

Methods. This study was based on national, population-based surveillance for invasive group A streptococcal (iGAS) disease. A case was defined as *S. pyogenes* isolated from blood or cerebrospinal fluid. Cases and the corresponding isolates were included during 1995-2007. Case-patients' 7-day outcome was obtained for 2004-2007. Isolates during 1995-2006 were T serotyped and during 2004-2007 *emm* typed. Additionally, all serotype T28 isolates since 1995 were *emm* typed. Isolates of an uncommon type *emm*84 were characterised by pulsed-field gel electrophoresis (PFGE) and superantigen profiling. Susceptibility to erythromycin, clindamycin and tetracycline was determined for all isolates during 2004-2007 and to levofloxacin during 2005-2007.

A case-control study of patients hospitalised for acute non-necrotizing cellulitis was conducted during April 2004-March 2005. Bacterial swab samples were obtained from skin lesions; blood culture samples were taken for detection of bacteraemia. Throat cultures of patients, family members and control subjects were assessed for pharyngeal carrier status. β-haemolytic streptococci and *Staphylococcus aureus* were isolated and identified; group A and G streptococci were analysed by T and *emm* typing and PFGE.

Results. During 1995-2007, the annual incidence of iGAS disease fluctuated (range by year, 1.1-3.9 cases per 100,000 population) but had an increasing trend, with peaks in 2002 (2.9) and in 2006-2007 (3.1-3.9). During 1998-2007, 1318 cases of iGAS were identified (55% in males), with an average annual incidence of 2.5 cases

per 100,000. Males had a higher incidence than females, especially among persons aged 45-64 years, while females had a higher incidence than males among persons aged 25-34 years. Occasional peaks of cases occurred during midwinter and midsummer. During 1995-2007, a total of 1457 iGAS isolates were analysed. The five most prevalent T types during 1995-2006 were T28 (29%), T1 (13%), TB3264 (12%), T12 (7%) and T8 (6%). The most common *emm* types during 2004-2007 were 28 (21%), 1 (16%), 84 (10%), 75 (7%), and 89 (6%). The prevalence of types T/*emm*1 and T/*emm*28 fluctuated during the study, with T/*emm*1 being the most predominant type in 1997-1998 and 2007 and T/*emm*28 in 1995-1996 and 2000-2006. Among T28 isolates, six different *emm* types were found during 1995-2006, with *emm*28 predominating. Among *emm*84 isolates, six PFGE strain types, with one dominating clone were found. Overall, 1.5% of the isolates were resistant to erythromycin, 0.5% to clindamycin and 16% to tetracycline. Females, especially of child-bearing age (15-44 years), had more infections by *emm*28 than males. The overall 7-day case fatality during 2004-2007 was 8%, peaking in 2005 (12%). Cases with *emm*1 infections were associated with higher than average case fatality (14%), whereas that of *emm*84 was 7%, and of *emm*28 only 2%.

A total of 90 patients with acute non-necrotizing cellulitis, 90 control subjects and 38 family members were recruited to a case-control study. β-haemolytic streptococci were isolated from 26 (29%) of 90 patients, either from skin lesions (24 patients) or blood (2 patients). Group G streptococcus (GGS, *Streptococcus dysgalactiae* subsp. *equisimilis*) was isolated most commoly (22%), followed by GAS (7%). GGS was also carried in the pharynx of 7% of patients and 13% of household members but was missing in control subjects. Several *emm* and PFGE types were found among the isolates. Six patients (7%) had recurrent infections during the study. In two patients, the same strain of GGS with identical *emm* and PFGE types was isolated from two consecutive episodes.

Conclusions. The incidence of iGAS disease had an increasing trend during the past ten years in Finland. Age- and sex-specific differences in the incidence rate and seasonal patterns were observed, and presumably differences in the predisposing factors and underlying conditions contribute to these distinctions. Changes in the *emm* type prevalence were associated with the increase in incidence and case fatality. The case fatality rate for *S. pyogenes* infections remained at a reasonably low level (8% overall) compared to that of other developed countries (mostly exceeding 10%). *emm* typing is sufficient for general epidemiological surveillance of iGAS disease, but for cluster or outbreak investigations, higher discriminatory power can be achieved when it is complemented by other techniques, such as PFGE.

In the case-control study, unexpectedly GGS, instead of GAS, predominated in acute non-necrotizing cellulitis. A predominance of GGS was also seen in the throat of case-patients and their family members, but not in control subjects. No clear predominance of a specific *emm* type was seen. The recurrent nature of cellulitis became evident.

This study adds to our understanding of the molecular epidemiology of *S. pyogenes* infections in Finland and provides a basis for comparison to other countries and future trends. Global *emm* type and outcome surveillance remain important in order to detect changes in the type distribution potentially leading to increasing incidence and case fatality.

Keywords: bacteraemia; cellulitis; *emm* typing; epidemiology; erysipelas; outcome; *Streptococcus dysgalactiae* subsp. *equisimilis*; *Streptococcus pyogenes*.

TIIVISTELMÄ

Tuula Siljander, Molecular and epidemiological aspects of *Streptococcus pyogenes* disease in Finland: Severe infections and bacterial, non-necrotizing cellulitis. [*Streptococcus pyogenes* -bakteerin aiheuttamien tautien molekyyliepidemiologiaa ja epidemiologiaa Suomessa: vakavat infektiot ja selluliitit] Terveyden ja hyvinvoinnin laitos, Tutkimus 23|2009. 160 sivua. Helsinki, Finland 2009. ISBN 978-952-245-174-3 (painettu); ISBN 978-952-245-175-0 (pdf)

Tausta ja tavoitteet. *Streptococcus pyogenes* (A-ryhmän streptokokki, GAS) aiheuttaa vakavuusasteeltaan vaihtelevia infektioita, lievistä hengitystieinfektioista vakaviin, invasiivisiin infektioihin, kuten bakteremioihin. Invasiivisia infektioita aiheuttavien A-ryhmän streptokokkikantojen vallitsevat tyypit vaihtelevat maantieteellisesti ja eri ajankohtina. Kansallinen seuranta osoitti invasiivisten streptokokki-tautitapausten lisääntyneen Suomessa vuonna 2006, ja hoitavat lääkärit huolestuivat kuolemaan johtavista vakavista taudinkuvista. Tämän johdosta käynnistettiin tutkimus taudin ilmaantuvuuden, kuolleisuuden ja bakteerityyppien viimeaikaisista suuntauksista. Bakteerin aiheuttama selluliitti ja erysipelas (ruusutulehdus) ovat paikallisia ja potentiaalisesti uusiutuvia ihon ja ihonalaiskudoksen infektioita. Tutkimuksen tavoitteena oli tunnistaa Suomessa selluliitti- ja erysipelasinfektioita aiheuttavia β-hemolyyttisiä streptokokkeja.

Menetelmät. Tutkimus pohjautui kansalliseen ja väestöpohjaiseen invasiivisten A-ryhmän streptokokkitautien seurantaan. Tapausmääritelmänä oli veren tai likvorin positiivinen *S. pyogenes* -viljelylöydös. Tapaukset ja niitä vastaavat bakteerikannat tutkittiin vuosilta 1995–2007. Tapauksiin liittyvä 7 päivän kuolleisuustieto oli saatavilla vuosille 2004–2007. Kannat vuosilta 1995–2006 T-serotyypitettiin ja vuosilta 2004–2007 *emm*-tyypitettiin. Lisäksi kaikki serotyyppiä T28 olevat kannat vuodesta 1995 asti *emm*-tyypitettiin. Harvinaista genotyyppiä *emm*84 olevat kannat karakterisoitiin käyttäen pulssikenttä-geelielektroforeesia (PFGE) ja superantigeenien määritystä. Kaikkien kantojen herkkyys erytromysiinille, klindamysiinille ja tetrasykliinille määritettiin vuosilta 2004–2007 ja levofloksasiinille vuosilta 2005–2007.

Tapaus-verrokkitutkimus, jossa tutkittiin akuutin selluliitin takia sairaalahoitoon joutuneita potilaita, toteutettiin aikavälillä huhtikuusta 2004 maaliskuuhun 2005. Ihorikkoalueelta otettiin bakteeriviljely tikkunäytteenä; veriviljelynäytteet otettiin bakteremian havaitsemiseksi. Potilaiden, perheenjäsenten ja verrokkien nielukantajuutta arvioitiin nieluviljelyiden perusteella. Näytteistä eristettiin ja tunnistettiin β-hemolyyttisiä streptokokkeja sekä *Staphylococcus aureus* -kantoja ja A- ja G-ryhmän streptokokit analysoitiin T- ja *emm*-tyypityksellä ja PFGE:llä.

Tulokset. Tutkimusjakson 1995–2007 aikana GAS-infektioiden ilmaantuvuus vaihteli (vuosittainen vaihteluväli 1,1–3,9 tapausta/100 000 henkilöä), mutta suuntaus oli nouseva ja huippuja esiintyi vuosina 2002 (2,9) ja 2006–2007 (3,1–3,9). Vuosien 1998–2007 aikana todettiin 1 318 invasiivista GAS-tapausta (joista 55 % miehiä) ja taudin keskimääräinen vuosittainen ilmaantuvuus oli 2,5 tapausta/100 000 henkilöä. Miehillä, etenkin 45–64-vuotiailla, oli korkeampi taudin ilmaantuvuus kuin naisilla, joilla taas 25–34-vuotiaiden ikäryhmässä oli korkeampi ilmaantuvuus kuin miehillä. Tapausmäärissä havaittiin satunnaisia huippuja keskitalvella ja keskikesällä. Kaikkiaan 1 457 invasiivista GAS-kantaa analysoitiin vuosina 1995–2007. Viisi yleisintä T-tyyppiä vuosina 1995–2006 olivat T28 (29 %), T1 (13 %), TB3264 (12 %), T12 (7 %) ja T8 (6 %). Yleisimmät *emm*-tyypit vuosina 2004–2007 olivat 28 (21 %), 1 (16 %), 84 (10 %), 75 (7 %) ja 89 (6 %). Tyyppien T/*emm*1 ja T/*emm*28 yleisyys vaihteli tutkimuksen aikana, T/*emm*1 oli yleisin tyyppi vuosina 1997–1998 ja 2007 ja T/*emm*28 vuosina 1995–1996 ja 2000–2006. T28-kantojen joukosta löytyi kuutta eri *emm*-tyyppiä vuosina 1995–2006, joista *emm*28 oli vallitseva. *emm*84-kantojen joukosta löytyi kuutta eri PFGE-kantatyyppiä, joista yksi vallitsi. Kaikista kannoista 1,5 % oli resistenttejä erytromysiinille, 0,5 % klindamysiinille ja 16 % tetrasykliinille. Naisilla, etenkin lapsensaanti-ikäisillä (15–44-vuotiailla), oli enemmän *emm*28-tyypin infektioita kuin miehillä. Keskimääräinen tapauskuolleisuus 7 päivän kohdalla oli 8 % vuosina 2004–2007, ja se nousi huippuunsa vuonna 2005 (12 %). *emm1*-tyypin infektioihin liittyi keskimääräistä korkeampi tapauskuolleisuus (14 %), kun *emm*84-tyypin infektioissa se oli 7 % ja *emm*28-infektioissa vain 2 %.

Kaikkiaan 90 selluliittipotilasta, 90 verrokkihenkilöä ja 38 perheenjäsentä rekrytoitiin tapaus-verrokkitutkimukseen. β-hemolyyttisiä streptokokkeja eristettiin 26 potilaalta 90:stä (29 %), joko ihorikosta (24 potilasta) tai verestä (2 potilasta). Potilasnäytteistä löytyi yleisimmin (22 %) G-ryhmän streptokokki (GGS, *Streptococcus dysgalactiae* subsp. *equisimilis*) ja seuraavaksi yleisimmin (7 %) A-ryhmän streptokokki. Potilaista 7 % ja perheenjäsenistä 13 % kantoi nielussaan G-ryhmän streptokokkia, kun taas verrokeilta sitä ei löytynyt. Kannat edustivat useita *emm*- ja PFGE-tyyppejä. Tutkimuksen aikana kuudella potilaalla (7 %) oli toistuva infektio. Kahdella heistä voitiin eristää kahdesta peräkkäisestä infektiosta sama identtisen *emm*- ja PFGE-tyypin omaava GGS-kanta.

Yhteenveto. Invasiivisten A-ryhmän streptokokki-infektioiden ilmaantuvuudessa oli nouseva suuntaus viimeisten kymmenen vuoden aikana Suomessa. Ilmaantuvuudessa ja vuodenaikaisvaihtelussa havaittiin ikä- ja sukupuolispesifisiä eroja, joihin vaikuttivat todennäköisesti erot potilaiden altistavissa tekijöissä ja perussairauksissa. Muutokset *emm*-tyyppijakaumassa liittyivät ilmaantuvuuden ja tapauskuolleisuuden nousuun. *S. pyogenes* -infektioihin liittyvä tapauskuolleisuus

pysyi kohtalaisen matalalla tasolla (keskimäärin 8 %) verrattuna muihin kehittyneisiin maihin (yleensä yli 10 %). *emm*-tyypitys on riittävä taudin yleiseen epidemiologiseen seurantaan, mutta ryppäiden ja epidemioiden selvittelyyn voidaan saada parempi erottelukyky kun menetelmää täydennetään muilla menetelmillä kuten PFGE:llä.

Tapaus-verrokki-tutkimuksessa selluliitti-infektioista löytyi yllättäen enemmän G- kuin A-ryhmän streptokokkibakteeria. G-ryhmän streptokokki vallitsi myös nielussa potilailla ja perheenjäsenillä mutta ei verrokkihenkilöillä. Mikään tietty *emm*-tyyppi ei ollut vallitseva. Tutkimus vahvisti käsitystä selluliitti-infektioiden toistuvasta luonteesta.

Tutkimus lisää tietoutta *S. pyogenes* -infektioiden molekyyliepidemiologiasta Suomessa ja luo pohjan vertailulle muiden maiden tilanteeseen ja tuleviin suuntauksiin. Maailmanlaajuinen *emm*-tyyppien seuranta ja kuolleisuustutkimukset ovat tärkeitä, jotta voidaan havaita sellaisia muutoksia tyyppijakaumassa, jotka voivat johtaa ilmaantuvuuden ja kuolleisuuden kasvuun.

Asiasanat: bakteremia; *emm-tyypitys*; epidemiologia, erysipelas; kuolleisuus, selluliitti; *Streptococcus dysgalactiae* subsp. *equisimilis*; *Streptococcus pyogenes*.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which shall be referred to throughout the text by Roman numerals given below (I-IV).

I Siljander T, Toropainen M, Muotiala A, Hoe NP, Musser JM, Vuopio-Varkila J. *emm*-typing of invasive T28 group A streptococci, 1995-2004, Finland. J Med Microbiol 2006; 55:1701-6.

II Siljander T, Lyytikäinen O, Vähäkuopus S, Säilä P, Jalava J, Vuopio-Varkila J. Rapid emergence of *emm*84 among invasive *Streptococcus pyogenes* infections in Finland. J Clin Microbiol 2009; 47:477-80.

III Siljander T, Lyytikäinen O, Vähäkuopus S, Snellman M, Jalava J, Vuopio-Varkila J. Epidemiology, outcome and *emm* types of invasive group A streptococcal infections in Finland (submitted).

IV Siljander T, Karppelin M, Vähäkuopus S, Syrjänen J, Toropainen M, Kere J, Vuento R, Jussila T, Vuopio-Varkila J. Acute bacterial, nonnecrotizing cellulitis in Finland: microbiological findings. Clin Infect Dis. 2008; 46:855-61.

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ABBREVIATIONS

APSGN	acute post-streptococcal glomerulonephritis
ARF	acute rheumatic fever
bp	base pair
CDC	Centers for Disease Control and Prevention (USA)
CFR	case fatality rate
CI	confidence interval
CLSI	Clinical and Laboratory Standards Institute (USA)
CSF	cerebrospinal fluid
emm	*emm* gene; M protein gene
GAS	group A streptococcus
GBS	group B streptococcus
GCS	group C streptococcus
GFS	group F streptococcus
GGS	group G streptococcus
HLA	human leukocyte antigen
iGAS	invasive group A streptococcus
KTL	National Public Health Institute of Finland (Kansanterveyslaitos)
MHC	major histocompatibility complex
Mga, *mga*	multiple gene regulator (gene)
MIC	minimal inhibitory concentration
MLST	multilocus sequence typing
NCBI	National Center for Biotechnology Information
NF	necrotizing fasciitis
NIDR	National Infectious Disease Register
NT	nontypable
OF	opacity factor
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
RR	rate ratio
rRNA	ribosomal RNA
SAg, SAgs	superantigen(s)
S. aureus	*Staphylococcus aureus*
Sic, *sic*	streptococcal inhibitor of complement (gene)
smeZ, *smeZ*	streptococcal mitogenic exotoxin Z (gene)
SLO	streptolysin O
SLS	streptolysin S
SNP	single nuclear polymorphism
SOF	serum opacity factor, also OF
Spe, *spe*	streptococcal pyrogenic exotoxin (gene)

S. pyogenes	*Streptococcus pyogenes*, also GAS
SSA, *ssa*	streptococcal superantigen (gene)
ST	sequence type
STSS	streptococcal toxic shock syndrome
subsp.	subspecies
TCR	T cell receptor
THL	National Institute for Health and Welfare (formerly KTL)
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

1 INTRODUCTION

Streptococcus pyogenes (group A streptococcus, GAS) is an organism that is able to cause a wide array of infections in humans. The disease manifestations can range in severity from mild upper respiratory tract infections and skin and soft tissue infections to the severe invasive infections, such as bacteraemia, pneumonia, necrotizing fasciitis, and streptococcal toxic shock syndrome. Infections caused by *Streptococcus pyogenes* are among the most ubiquitous which make this a very important human pathogen.

S. pyogenes has complex virulence mechanisms, which contribute to its efficiency as a pathogen. The major virulence factor of GAS is the M protein, which is expressed in abundance on the bacterial surface and encoded by the *emm* gene. The outer part of the M protein is extremely variable, and this hypervariability has been used as the basis for a serological typing method (M typing) since the 1960s [187]. Currently, the most widely used typing method of GAS is based on sequencing the hypervariable part of the *emm* gene. Well over a hundred distinct *emm* sequence types have been identified among GAS strains [17, 100, 101].

The majority of GAS infections are mild pharyngeal infections and skin infections. Tens of thousands of streptococcal pharyngitis cases occur annually in Finland. In contrast, severe streptococcal diseases occur only in 1-10 persons per 100,000 population in developed countries annually (currently around 200 cases per year in Finland). Attempts to control and eradicate these infections are important for several reasons. Firstly, mild infections can lead to a more severe disease, and patients with mild infections can transmit the bacteria to others. Although the severe GAS diseases are relatively rare, they constitute a major global burden, resulting in hundreds of thousands of cases each year, most of which occur in less-developed countries [43]. Secondly, despite antimicrobials and other medical treatment, severe infections are associated with a high mortality rate even exceeding 40% [244]. Group A streptococcus has been estimated to be among the ten most common causes of death due to individual pathogens globally [43]. A quarter to a fifth of cases have no predisposing factors to infection, and the infections can affect people of all ages [185]. These characteristics also contribute to fear and anxiety in the general public and media, referring to this pathogen as “the killer bug” or “the flesh-eating bacterium”.

In the late 1980s, a change in the epidemiology of severe group A streptococcal infections, with an increase in the incidence and severity of disease, was documented by many countries [140, 157, 270, 295, 300]. The role and pathogenic potential of especially type M1, but also of M3, in the epidemics were of specific

interest [53, 204, 300]. The incidence of invasive GAS (iGAS) disease typically varies by time and geographic region, and this presumably reflects a population's susceptibility to particular strains but also the natural variation in the predominant types [242]. Variation in the type distribution may also lead to fluctuation in the severity of infections and mortality rates.

Bacterial erysipelas and cellulitis refer to acute, diffuse, spreading infections of the skin and subcutaneous tissue. Group A streptococcus has been considered the main causative agent of erysipelas and cellulitis, although streptococci of other groups can also cause these infections [37, 49, 94]. Although erysipelas and cellulitis are usually not life-threatening infections, they have an unfortunate tendency to recur and therefore cause remarkable morbidity, especially in elderly patients [31, 94, 116, 162]. Pharyngeal streptococcal colonisation might be a risk factor for a symptomatic infection. Many other general and local risk factors and individual differences in immune response can influence the susceptibility to infections and to recurrences. Distinguishing between erysipelas and cellulitis may be impossible based only on the clinical picture of the infection [31].

Molecular epidemiology focuses on characterisation of molecular properties of the causative pathogen in infectious diseases. Surveillance is of importance in order to rapidly detect changes in type distribution or resistance to antimicrobials. Several approaches for developing an effective and safe vaccine against *S. pyogenes* infections have been made, but vaccine development for this organism has proven very challenging. Identification of strain types also serves as a basis for studies of disease pathogenesis and vaccine development [244].

2 REVIEW OF THE LITERATURE

2.1 Human pathogenic β-haemolytic streptococci

Streptococci are gram-positive, facultatively anaerobic cocci-shaped bacteria, which occur in pairs or chains and include many different species (including *Streptococcus pyogenes*) infecting humans and animals. The name *Streptococcus* literally means a chained round-shaped bacteria and *pyogenes* refers to pus formation by this bacterium. The classification of streptococci is based on their haemolysis - α, β, and γ - on blood agar plates, which result in partial, complete, or no lysis of red blood cells, respectively. β-haemolysis shows on a blood agar plate as a clear zone around the bacterial colonies. Dr. Rebecca Lancefield was the first to develop a method for classification of β-haemolytic streptococci by serologically detecting their group-specific polysaccharide antigens [186]. The human pathogenic β-haemolytic streptococci generally include the Lancefield serogroups A, B, C and G [99]. The distinction of serogroups does not fully follow the species determination of streptococci, as is shown in Table 1, which outlines the taxonomy within these Lancefield groups. Rarely, strains of *S. dysgalactiae* subsp. *equisimilis* possess the group A antigen. Besides the species covered by the table, many other streptococcal species causing infections in animals exist.

Table 1. The most important human pathogenic β-haemolytic streptococci (adapted from [99])

Species	Lancefield group	Origin
Streptococcus pyogenes	A	human
S. agalactiae[a]	B	human, bovine
S. dysgalactiae subsp. *equisimilis*	A, C, G, L	human, animals
S. equi subsp. *zooepidemicus*	C	animals, human
S. canis	G	dog, human
S. anginosus (group)[b]	A, C, G, F, none	human
S. constellatus subsp. *pharynges*	C	human

[a] *S.* is abbreviation for *Streptococcus*.

[b] The *S. anginosus* group includes β-haemolytic strains of *S. anginosus*, *S. constellatus* and *S. intermedius*.

The β-haemolytic streptococci of groups A, C and G colonise humans and cause clinically similar mild and more severe infections. In particular, bacteraemic strains of group G and C streptococcus (*S. dysgalactiae* subsp. *equisimilis*) seem to share characteristics of pathogenic potential with group A strains [99, 114]. In contrast, group B streptococcus (GBS) has traditionally caused infections predominantly in neonates and pregnant women but these infections have lately increased also among nonpregnant adults with underlying diseases and the elderly [105, 269].

2.2 *Streptococcus pyogenes*; group A streptococci

2.2.1 Carriage and transmission

Streptococcus pyogenes is strictly a human pathogen [294]. It can be carried asymptomatically on the skin (superficial layers of the epidermis) and mucous membranes, such as the oropharyngeal mucosal epithelium, the nasal epithelium (although less commonly), the genital tract, and the perianal area [13, 294]. A pharyngeal GAS carrier can be defined as an asymptomatic individual with a positive throat culture and no active immune or inflammatory response, or an asymptomatic child who tests positive for GAS after completing accurate antimicrobial treatment of GAS pharyngitis [205, 311]. The latter definition refers to chronic carriage.

School-aged children (5-15 years) are considered as the major reservoir of GAS, with a prevalence of pharyngeal carriage of 15-25% or more depending on the study setting [55, 128, 205, 311]. Adults, in contrast, have low pharyngeal carrier rates (<5%) [294]. The carrier state may vary by age, season, and geographical location [128, 205]. Carriage can be transient or persistent, and the carried strain can be replaced by a new type of GAS [205, 311]. Internalisation of GAS into epithelial cells, protecting the pathogen from antimicrobial treatment, has been suggested as a mechanism for persistent throat carriage [247, 271].

The bacteria can be transmitted from carriers and especially those with pharyngeal disease to other persons and the surroundings by direct contact with respiratory secretions or by aerosolised droplets [55, 137, 205, 294]. Crowded living conditions, such as day-care centers or military settings, favour the transmission of the bacteria [137, 277].

2.2.2 Diseases caused by *S. pyogenes*

Streptococcus pyogenes causes a wide array of disease manifestations ranging in severity. Diseases presumably caused by *S. pyogenes* (and other streptococcal species) were already described in the 18th and 19th century, long before there was any knowledge of streptococci as the causative agent, or that many of the different disease manifestations were actually caused by the same organism. In 1874, Theodor Billroth demonstrated the existence of streptococci in patients with erysipelas and wound infections and was the first to use the term streptococcus [3, 77].

The most common superficial infections caused by GAS are upper respiratory tract infections, including acute tonsillitis (“strep-throat”) or pharyngitis [32, 77]. GAS

can also cause otitis media and sinusitis. Scarlet fever is caused by strains of *S. pyogenes* which express one or more of certain pyrogenic exotoxins (A, B or C), and this disease is thus accompanied by toxin-mediated symptoms, such as a characteristic rash, "strawberry" tongue, and desquamation of the skin [64]. Scarlet fever used to be a life-threatening infection before the era of antimicrobials, commonly causing epidemics with a high mortality, but it is now a milder disease, most frequently presented by a pharyngitis accompanied by the distinctive rash [173, 293].

S. pyogenes causes a wide variety of skin and soft-tissue infections. Impetigo (or pyoderma) is a superficial, localised and purulent infection of the dermis and epidermis. It is more common in humid and warm climates, usually occurring in children, and usually on exposed areas such as the face, hands or feet [31, 197]. Erysipelas and cellulitis, which are more deeply situated non-necrotizing infections of the skin and underlying tissue, are discussed in more detail in section 2.2.3. Necrotizing fasciitis (NF) is a severe infection of the deeper subcutaneous tissue and fascia [304]. It presents with severe local pain and rapid tissue destruction and leads to systemic symptoms, which may include shock and multiorgan failure, and subsequently, death.

In addition to the above-mentioned infections, *S. pyogenes* can also cause other localised infections, such as meningitis, peritonitis, pneumonia, septic arthritis, and puerperal sepsis (see also section 2.3.4.). Puerperal sepsis, an infection predominantly caused by GAS and also known as "childbed fever", was formerly a much dreaded disease and a common cause of death for young women in Europe in the 18th and 19th centuries [7, 77]. Epidemics with a high mortality (50% or more) were commonly seen in maternity wards due to transfer of bacteria to women by the hands of attending physicians, who had previously been performing autopsies or examining other patients [3, 7, 77]. One contributory factor to the epidemics was the fundamental change in the society towards giving birth in large maternity hospitals instead of at home [7]. These days, with the use of aseptic techniques and treatments available, the mortality due to puerperal sepsis is estimated at 3.5% or less, although outbreaks still occur [51].

A focal infection may or may not be associated with bacteraemia, which by definition means the presence of cultivatable bacteria in the bloodstream, a state that may also be transient and inconsequential, in contrast to sepsis, which refers to the body's systemic response to infection [227]. On occasion, the focus of the infection cannot be identified, with the only disease manifestation being bacteraemia due to *S. pyogenes*. Some of the infections by *S. pyogenes*, especially NF, are associated with a most severe complication, streptococcal toxic shock syndrome (STSS), which

involves hypotension, shock, and multiorgan failure and consequently leads to high mortality [1]. STSS and scarlet fever can be regarded as toxin-mediated diseases caused by *S. pyogenes* [88].

On occasion, GAS infection can be followed by an inappropriate immunologically-mediated response and tissue-specific damage. These non-suppurative complications of *S. pyogenes* include acute post-streptococcal glomerulonephritis (APSGN) and acute rheumatic fever (ARF). ARF is a sequela of an untreated infection of the upper respiratory tract, and it can lead to the development of rheumatic heart disease, whereas APSGN can also be associated with a skin infection [88, 203]. The clinical manifestations of APSGN or ARF occur approximately 3 weeks after the underlying infection, and they are caused by the so-called nephritogenic or rheumatogenic strains, respectively [88, 145, 203]. These post-infectious streptococcal diseases are more common in developing countries, where rheumatic heart disease is the most common cardiac disease in children and young adults [43]. These infections used to be more common in the developed countries, such as the USA, but their prevalence has markedly decreased during recent decades [278].

Table 2 presents the classification of *S. pyogenes* diseases and streptococcal toxic shock syndrome, as originally defined by the USA Working Group and used by many countries for surveillance purposes [1]. The classification includes a division of diseases caused by *S. pyogenes* into invasive and noninvasive, where invasive disease refers to isolation of *S. pyogenes* from a normally sterile site. The term "severe GAS diseases" may also be used and it can be regarded to include invasive disease, acute rheumatic fever, rheumatic heart disease, and acute post-streptococcal glomerulonephritis [43].

Table 2. Classification of streptococcal infections (adapted from [1])

I. Streptococcal toxic shock syndrome (STSS)[a]
   1. Isolation of *S. pyogenes* from a normally sterile site or from a nonsterile site
   2. Clinical signs of severity
      A. Hypotension, and:
      B. ≥2 of the following signs: renal impairment, coagulopathy, liver involvement, adult respiratory distress syndrome, erythematous rash, soft-tissue necrosis including NF

II. Other invasive infections: isolation of *S. pyogenes* from a normally sterile site in patients not meeting criteria for STSS
   A. Bacteraemia with no identified focus
   B. Focal infections with or without bacteraemia

III. Scarlet fever: a scarlatina rash with evidence of *S. pyogenes* infection such as pharyngotonsillitis

IV. Noninvasive infections: isolation of *S. pyogenes* from a nonsterile site
   A. Mucous membrane
   B. Cutaneous

V. Nonsuppurative sequelae
   A. Acute rheumatic fever
   B. Acute glomerulonephritis

[a] A definite or probable case of STSS depending on fulfilling the criteria.

2.2.3 Streptococcal non-necrotizing cellulitis

Bacterial non-necrotizing cellulitis refers to an acute, diffuse, spreading infection of the skin and subcutaneous tissue, excluding cutaneous abscesses, necrotizing fasciitis, septic arthritis, and osteomyelitis [296]. Cellulitis usually refers to a more deeply situated skin infection, with a diffuse swelling and reddening of the skin without a clear boundary, whereas erysipelas can be considered as a superficial form of cellulitis, usually being manifested by a well-demarcated erythema of the skin. The distinction between cellulitis and erysipelas is not clear-cut, and these conditions share typical clinical features – for example, local signs of inflammation and sudden onset, usually with a high fever [31]. If the clinical diagnosis is not always straightforward, neither is the terminology. The terms erysipelas and cellulitis are used inconsistently, partly due to the customary use of the term erysipelas, especially in some parts of Europe, to describe both infections, whereas in the USA only the term cellulitis is used to cover both infections. In this book, the term cellulitis is used to encompass all non-necrotizing cellulitis and erysipelas infections caused predominantly by streptococci if not otherwise specified.

S: pyogenes has been considered to be the main causative agent of erysipelas and cellulitis, although streptococci of group G and C (most importantly, *Streptococcus dysgalactiae* subsp. *equisimilis*), group B, and rarely, staphylococci can also be involved in these infections, sometimes concomitantly with streptococci [35, 37, 48, 94, 148]. The role of *Staphylococcus aureus* as a causative pathogen is believed to be larger in cellulitis as compared to classic erysipelas infections [304]. Colonisation of the skin by the pathogen is frequently observed during the infection [37, 161]. Patient blood cultures are positive for β-haemolytic streptococci in only <5% of cases [31, 37, 48, 94, 161]. The role of streptococcal toxins contributing to local inflammation is probably very important in the pathogenesis of this disease [37].

The predominant infection site is the legs; the face or arms are more rarely affected [37, 48]. Known risk factors for infection include lymphoedema and, most notably, disruption of the cutaneous barrier, such as local trauma, leg ulcer, toe-web intertrigo, and chronic fungal infections, which provide a site of entry for the pathogen(s) [35, 37, 87, 133, 223, 265]. A portal of entry is mostly found but it is not always possible to affirm it as such [48, 87]. Other local risk factors are lymphatic congestion, venous or arterial insufficiency, and a previous history of a cellulitis infection [35, 162]. Among the general risk factors, being overweight, diabetes, smoking, and alcoholism are worth mentioning although they have been variably identified depending on the study [48, 87, 162, 264]. In some studies, a slight male predominance has been identified in patients with cellulitis [35, 191, 223].

In Finland, the recommended antimicrobial treatment for streptococcal cellulitis is penicillin administered either intravenously or intramuscularly, and after the recovery has started (usually after 3-5 days), followed by oral administration for a total of three weeks [85]. For patients who are penicillin-allergic, cephalosporins are recommended, or, alternatively, clindamycin in cases with severe penicillin allergy. Antimicrobials other than penicillin are needed for infections where *Staphylococcus aureus* is suspected or confirmed, either as the sole pathogen or concomitantly with a streptococcus [31, 37, 85]. An Italian study suggested that the course of polymicrobial cellulitis may be more severe than monomicrobial infection, with these cases more likely to have open skin lesions with a heavier bacterial load in the infection site and to require a longer stay in hospital [190].

Twenty to 30% of cellulitis patients have a recurrence within a 3-year follow-up period [94, 162]. According to some studies, recurrences share mostly the same risk factors as the first episodes of infection, identified as lower extremity oedema, high body mass index, and smoking [191, 194]. Usually after a certain number of recurrences, long-term prophylaxis with daily oral penicillin or monthly benzathine

penicillin injections is initiated [37, 48]. It is also important to treat the portal of entry, most often the toe-web intertrigo or chronic mycosis, to prevent further recurrences [62, 87, 265]. Anal colonisation with streptococci may also constitute a reservoir for relapsing cellulitis infections [37, 95]. Although cellulitis infections are usually not life-threatening, they cause remarkable morbidity especially in the elderly patients and considerable costs arise from hospitalisation [31, 116].

2.2.4 Virulence factors

S. pyogenes has very complex virulence mechanisms, which is illustrated by the fact that more than 60 properties relating to this pathogen's virulence have been described so far [213]. The qualities that constitute a virulence factor include those which confer antiphagocytic properties, adherence to epithelial cells, internalisation into cells, invasion, spread through tissues or systemic toxicity [34]. Nearly all surface components of GAS have been suggested to be virulence-associated, but the most important structures for virulence seem to be the capsule and M proteins, as discussed next [152].

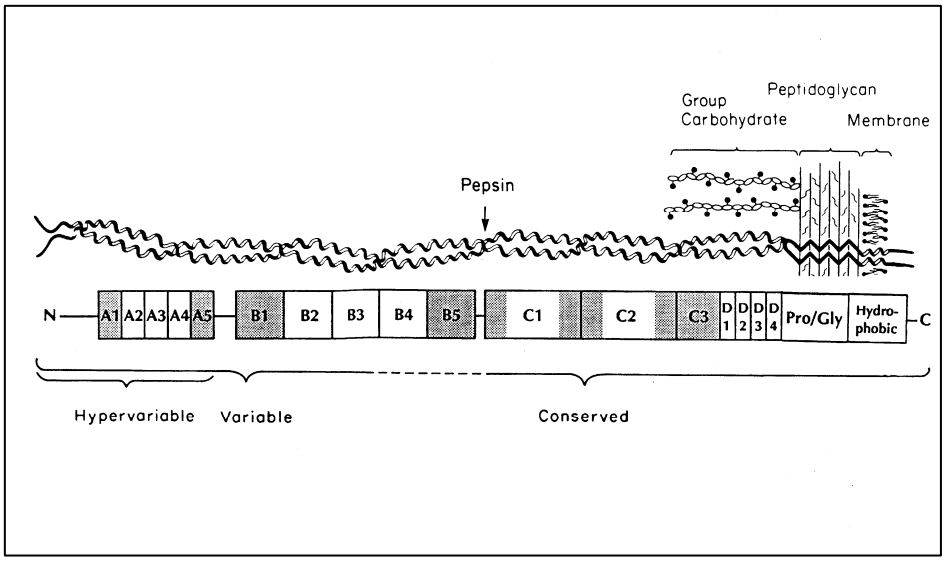
As the first line of defence, group A streptococci have a hyaluronic acid capsule, which is poorly immunogenic, protects the pathogen from phagocytosis, and is involved in adherence and invasion [65, 329]. Streptococcal strains vary greatly in their degree of capsular expression, and very mucoid strains with heavy encapsulation have been linked more often to invasive infections as well as ARF [34, 299]. The cell wall consists of peptidoglycan with lipoteichoid acid components, which may also play a role in the pathogenesis by binding to fibronecting and thus facilitating the adherence to pharyngeal epithelial cells [34]. It is not clear if the group carbohydrate, which is attached in the cell wall and confers the serologic group specificity, has a role in pathogenesis.

M protein, encoded by the *emm* gene, is the most abundant surface protein of streptococci and the major virulence factor of GAS. Strains not expressing M protein are non-virulent [33]. M protein is a highly polymorphic protein of 41-80 kDA in size, and its structure resembles that of the staphylococcal A protein. It has a dimeric α-helical coiled-coil structure, which forms fibres (of 50-60 nm in length) that protrude outwards from the cell wall (Figure 1) [109]. The N-terminal outer part of the protein has a hypervariable sequence, while the C-terminal part, associated to the cell wall and membrane, is conserved.

The α-helical structure of the protein consists of a repeating seven-residue amino acid pattern, which tolerates a considerable amount of variation in the primary sequence. The amino acid variation, resulting in irregularity and instability of the

coiled-coil structure, has been found to be a requisite for virulence [214]. The protein also contains a series of tandem repeat regions (A-C) of different sizes, which vary by M type. The hypervariability in the aminoterminal part forms the basis for M and *emm* typing, which are described in more detail in section 2.2.6. In addition to GAS, group C and G streptococci infecting humans also harbour heterogeneous M proteins [33, 41, 58].

Figure 1. A schematic representation of the streptococcal M protein. Adapted from [109, 110].



M proteins are part of a protein family of M-like proteins, which have homology to several human proteins, such as the heavy chain of cardiac myosin, type I keratins, and human α-tropomyosin [109]. Many GAS strains possess more than one M-like protein, encoded by *emm*-like genes in the Mga (multiple gene regulator) regulon (also discussed later in this section). There are four major subfamilies of *emm* genes, and the chromosomal arrangement of them reveals five major *emm* patterns A-E [28, 27, 210]. These patterns can act as genotypic markers for tissue-site preferences among *S. pyogenes* strains. Studies suggest that horizontal transfer events of *emm*-like gene sequences between strains of *S. pyogenes* have commonly occurred, contributing to the evolution of these genes [331, 330]. There is indeed evidence that the evolution of the transcription regulatory gene *mga* (multiple gene regulator) is linked to the tissue tropism (niche specialisation) of *S. pyogenes*, which may explain the associations of *emm* types to certain disease manifestations (see also section 2.3.6) [28]. The M proteins are involved in the virulence of GAS infections by many different mechanisms, which are described next.

Group A streptococci are primarily located in the extracellular space during infections, although they can also exist intracellularly within phagocytic cells [317]. The main host defences against the pathogen are the complement, the antibodies, and the phagocytic cells, which are targeted at extracellular pathogens [152]. Complement is the primary host defence against pathogens in the human blood. It is a system consisting of several soluble serum proteins and regulatory proteins that form part of the innate (non-adaptive) immune response system. The activation of either the classical or the alternative pathway of complement leads to the opsonisation (coating) of the target with complement protein C3b. The M protein interferes with this defence mechanism by resisting opsonisation and the subsequent phagocytosis and killing by human leukocytes [34]. Several mechanisms contribute to this antiphagocytic effect. Most importantly, the M protein's ability to bind complement regulatory proteins, the C4b-binding protein and Factor H, interferes with the effect of C3b and phagocytosis [34, 152]. Furthermore, the binding of M protein to human fibrinogen may sterically interfere with the binding of complement protein C3b to the bacterial cell surface [34]. This results in resistance to phagocytosis and the complement's ineffectiveness in direct killing of GAS by production of membrane attack complexes on the surface of the pathogen.

Other functions of the M protein contributing to virulence are the adherence to skin keratinocytes and involvement in the internalisation and invasion of human cells [34]. M protein has also been found to form complexes with fibrinogen, which by binding to intergrins on the surface of neutrophils are able to activate the release of heparin binding protein, an inflammatory mediator inducing vascular leakage [131]. STSS is characterised by excessive plasma leakage and M protein/fibrinogen complexes have been identified in tissue biopsies from an NF patient with STSS [131]. Furthermore, M protein can interact with human toll-like receptor 2 and stimulate monocytes to produce high amounts of proinflammatory cytokines, a process that is particularly enhanced by heparin binding protein [249].

Quite recently, a pilus-like structure, containing T antigens of the Lancefield T typing system, was recognised on the surface of GAS, indicating that at least some of the T proteins constitute a pilus structure [225]. Pili are known to be virulence factors in gram-negative bacteria and may serve the same function in GAS [26]. The pilus components are members of a family of extracellular matrix-binding proteins involved in adhesion and invasion. The variability in the T proteins is also used as a basis of serological typing of streptococci (T typing) [224].

Group A streptococci have several fibronectin-binding surface proteins. One of these is protein F1 (PrtF1, also known as SfbI, streptococcal fibronectin binding protein I), the expression of which is enhanced in an oxygen-rich environment and

thus it is thought to be important for adherence to mucosal or skin surfaces and for internalisation [34, 126]. A homologous protein has been identified in group G streptococcus (GGS), indicating that a horizontal transfer of genes has taken place between group A and G streptococci [320]. The serum opacity factor (SOF, OF) is a streptococcal surface protein that binds to fibronectin and fibrinogen, and contributes to the pathogenesis of GAS by promoting invasion to human epithelial cells [61, 318].

Several extracellular secreted proteins also contribute to virulence. Secreted deoxyribonucleases (DNases) A, B, C, and D, enzymatically participate in the degradation of DNA, possibly facilitating the spread of streptococci through tissues [34]. An extracellular DNase also protects from phagocytosis and thus enhances the evasion from human immune functions [302]. The streptokinase enzyme is produced by all strains of GAS and proteolytically converts plasminogen to active plasmin, a process that contributes to the dissolution of clots [34]. A hyaluronidase enzyme is able to degrade hyaluronic acid in human connective tissue [34]. Yet another protein, a C5a peptidase, specifically cleaves the human chemotaxin C5a, inhibiting chemotaxis and preventing phagocytosis. Many invasive GAS strains also express a nicotine-adenine-dinucleotidase (NADase), but its function in pathogenesis is not known [294].

Some secreted proteins work against the complement's function. Streptococcal inhibitor of complement (Sic) is produced predominantly by M1 strains and it inhibits the function of the complement membrane attack complex and prevents the bacterial cell from lysing [106]. Sic also interacts with a protein ezrin that is located in the eukaryotic plasma membrane and inhibits the human polymorphonuclear cells from internalising the microbe, enhancing the pathogen's survival [138]. The *sic* gene coding for the Sic protein is located in the Mga regulon near the *emm* gene [4]. Sic is a highly variable protein and there is evidence that Sic variants are being selected on the human mucosal surface [139].

Toxins have a critical role in streptococcal pathogenesis by contributing to the severity of infection [177, 234]. Secreted toxins and enzymes facilitate pathogenesis and invasion to tissues. Two distinct haemolysins have been found: streptolysin O (SLO) and streptolysin S (SLS), which cause β-haemolysis of blood. SLO is a pore-forming cytolysin which has toxic effects on a variety of cells and is able to induce apoptosis of macrophages [319]. SLO is antigenic and produced by almost all GAS strains as well as by some group C and G strains [34]. SLS is one of the most potent cytotoxins inhibited by phospholipids, such as serum lipoproteins. SLS and SLO are both able to mediate damage to the membranes of polymorphonuclear leukocytes [34].

The streptococcal superantigens (SAgs) are able to induce potent inflammatory responses [177]. The SAgs are the streptococcal pyrogenic exotoxins (Spes) A, C, G-M, the streptococcal superantigen (SSA) and the streptococcal mitogenic exotoxin SmeZ [60]. Normally, antigens are processed into small peptides by the antigen presenting cells and displayed on the surface of these cells bound as complexes to major histocompatibility complex (MHC) class II molecules. The MHC-peptide complexes are then recognised by T cells via T cell receptors (TCR), which induces T cell activation and leads to the release of inflammatory cytokines. However, superantigens are able to induce T cell activation without prior processing in the antigen presenting cells by binding directly to MCH class II molecules and TCRs outside the antigenic binding site [130, 177]. The binding is not dependent on the antigen specificity of the T cell, and SAgs may stimulate as many as 20% of all T cells, whereas the binding of conventional antigens activates approximately 0.01% of T cells. SAgs are believed to have a critical role in NF and STSS [34, 177, 239]. The excessive proliferation of T cells and the subsequent massive release of pro-inflammatory cytokines and interleukins is believed to lead to capillary leak, and to be responsible for the most severe consequences as seen in STSS: hypotension, shock, multi-organ failure, and death [1, 292]. The lack of protective anti-SAg antibodies has been found to be associated with an increased risk for developing STSS [15, 97].

The distribution of the streptococcal SAgs varies among strains [11]. There are three chromosomally encoded (*speG*, *speJ* and *smeZ*) and eight prophage associated (*speA*, *speC*, *speH*, *speI*, *speK/L*, *speL/M*, *speM* and *ssa*) superantigen genes [201, 231]. Some of the streptococcal superantigens have homology to staphylococcal superantigens, indicating a horizontal transfer from *S. aureus* [253]. The SpeA exotoxin has been associated with severe scarlet fever cases [294]. There is strong support to the hypothesis of SpeA enhancing the persistence of GAS in natural populations [21]. Strains harbouring *speA* and/or *smeZ* genes are potentially involved with severe disease and STSS [22, 97, 201, 230, 254, 295]. *SmeZ* exhibits the highest allelic variation of SAgs and especially its variant *SmeZ-2* is the most potent superantigen [253]. The majority of strains seem to harbour *speG* gene [72, 90, 198]. There is evidence for identical or nearly identical SAg genes of *speM*, *ssa,* and *smeZ* existing in *S. dysgalactiae* subsp. *equisimilis* and *S. canis* strains, suggesting a frequent interspecies gene exchange between these species and *S. pyogenes* [149, 163].

The gene encoding SpeB is present in virtually all GAS strains, but its expression varies greatly from strain to strain [294]. Although SpeB was initially believed to be a superantigen, its toxic potential may be solely due to its cysteine protease activity [34]. SpeB contributes to pathogenesis in several ways, resulting from its ability to

cleave a variety of host proteins, such as immunoglobulins, vitronectin, and fibronectin, and to induce the release of biologically active peptides which promote the pathogen's ability to spread through tissues [34]. Similarly, SpeF toxin is not a superantigen but a streptococcal DNase [253].

Novel streptococcal superantigens are continuously being found. The fact that M1 serotype strains are overrepresented in STSS has inspired researchers to investigate these strains in more detail. There is recent evidence that a soluble M1 protein is actually a novel streptococcal superantigen [248]. It remains to be seen if this is a unique property of the M1 protein, as the distinct M proteins do share high homology with each other at a structural level.

Although certain SAg profiles are found more frequently among invasive isolates, no clear association has been found between pathogenicity and the presence of single SAg genes [49, 72]. However, pharyngeal isolates may harbour SpeA and SpeC genes more often than the invasive isolates [128]. Superantigen genes are not randomly distributed among GAS isolates, but have been found to associate with particular M/*emm* types [60]. However, the occurrence of SpeA in isolates of the same M type varies depending on the geographic region [50, 228, 233]. Several toxin-gene profiles can be identified within a single *emm* type, but mostly 1-2 toxin-gene profiles dominate, indicating that a few successful invasive clones have spread throughout the world [268].

One important factor contributing to the pathogenesis and host adaptation is the allelic variation of genes encoding virulence factors, as many of the GAS virulence genes are polymorphic [257]. The regulation of gene expression is also likely to contribute to GAS survival. A recent functional analysis showed that a very effective adaptative metabolic shift occurs within 30 minutes of bacterial exposure to human blood, manifested by the increased transcription of genes encoding for superantigens and host-evasion proteins [118]. The first virulence network that was described in GAS was the Mga, the multiple gene regulator of GAS. The Mga-regulated genes encode, in addition to the M protein and M-like proteins, the streptococcal collagen-like protein, the SOF, the C5a peptidase, and Sic, among others [64, 258]. There is suggestive evidence that the Mga-regulated products are required in adhesion, internalisation, and immune evasion, in other words, during entry of GAS into new tissue sites [180, 212].

2.2.5 The GAS genome

The group A streptococcal genome is a single circular chromosome, approximately 1.9 Mb in size. Approximately 10% of the genome consists of variable regions, including prophage-like elements or their remnants and insertion elements, for which prophages may be the primary source [11]. The rest, roughly 90% of the genome, is called the "core genome", which is the part of the genome not including prophage-like and obvious insertion elements. The core genome encodes for many proven or putative virulence factors such as M protein, streptolysin O, streptolysin S, streptococcal cysteine protease, and the hyaluronic acid capsule [20].

GAS is unique among the bacterial species so far sequenced in the magnitude to which the phages account for genome diversification and variation of gene content. Acquisition and loss of prophages generates distinct genotypes with novel combinations of virulence factor genes [22]. Most of the SAg genes are associated with the prophage sequences, except for *speG*, *speJ,* and *smeZ*, which are encoded by the core genome [253]. The prophage elements and virulence genes can be horizontally transferred between strains of GAS and also between different streptococcal species, which may lead to clones with enhanced potential for pathogenesis [5, 75, 164].

At present, the genome of 13 GAS strains of 10 M types have been sequenced (Table 3) and more genomic sequences are in progress according to information from NCBI (National Center for Biotechnology Information) GenBank [21, 217, 232]. The size of the sequenced genomes varies from 1.815,783-1.937,111 bp depending on the strain. The sequenced genomes are not closely related to each other but have been selected for their properties, e.g. M type, virulence or source of isolation. All of the sequenced strains are polylysogenic (including multiple prophages) and the prophages constitute the primary source of variation in these strains [11]. Each prophage encodes for 1-2 virulence factors.

Table 3. The sequenced Streptococcus pyogenes genomes. Adapted from [21, 217].

Strain	M type	No. of pro-phages	GenBank accession no.	Information on the strain, association with disease	Reference
SF370	M1	4	AE004092	wound infection	[107]
MGAS5005	M1	3	CP000017	cerebrospinal fluid (Canada)	[301]
MGAS10270	M2	5	CP000260	pharyngitis (USA)	[20, 21]
MGAS315	M3	6	AE14074	STSS, high virulence (USA)	[23]
SSI-1	M3	6	BA000034	STSS (Japan)	[231]
MGAS10750	M4	4	CP000262	pharyngitis (USA)	[20, 21]
Manfredo	M5	5	AM295007	acute rheumatic fever	[141]
MGAS10394	M6	8	CP000003	pharyngitis, paediatric, macrolide- resistant (USA)	[12]
MGAS2096	M12	2	CP000261	acute glomerulonephritis (Trinidad)	[20, 21]
MGAS9429	M12	3	CP000259	pharyngitis, paediatric (USA)	[20, 21]
MGAS8232	M18	5	AE009949	acute rheumatic fever (USA)	[288]
MGAS6180	M28	4	CP000056	invasive infection (USA)	[121]
NZ131	M49	3	CP000829	acute glomerulonephritis (New Zealand)	[217]

Sequencing of the genomes has revealed that variably-present genes are confined to few genomic areas, mostly located in the middle of the chromosome, distal to the origin of replication. Different GAS strains may include the same foreign genetic elements but these may be inserted in different locations in the genomes, which adds complexity to the genomic research of streptococci [21]. Considerable variation exists in the prophage content and prophage-associated virulence factors among strains of the same M type, in other words, strains of the same M type are not clonally related [11, 21, 23, 22, 122, 121, 301]. *emm*28 isolates have been shown to have considerable diversity in the prophage-associated virulence gene content, as compared to *emm*1 isolates, the majority of which are thought to be descendants of a virulent clone that emerged and became abundant during the 1980s [98, 122, 301].

In addition to prophage sequences, unique genetic material encoded by integrated conjugative elements (ICEs), which may influence the fitness or survival properties of the pathogen, has recently been found in the GAS genome [217]. The known and putative virulence factors associated to non-bacteriophage related genes have been found to be restricted to 11 genomic loci which are generally accompanied by mobile genetic elements [213]. Profiling of these loci could be a useful typing tool in epidemiological studies of GAS.

Sequencing of the type M28 genome has given new insight into puerperal sepsis. A non-prophage region of 37.2 kbp in size and encoding for seven inferred extracellular proteins seems to be present in all M28 strains. Interestingly, RD2 has a similar composition and sequence as regions of certain serotypes of group B streptococci, the primary causative agent of neonatal infections due to maternal epithelial colonisation and transfer during pregnancy or delivery [121, 337]. Suggestive evidence points to aquisition of RD2 with horizontal gene transfer from GBS to GAS, which could have enhanced the pathogenic potential and niche adaptation of the M28 strains, contributing to their overrepresentation in neonatal invasive infections and puerperal sepsis [122, 121, 289]. One of the extracellular proteins encoded by RD2 is the R28 protein, which promotes the adhesion of GAS to human epithelial cells in laboratory studies and possibly also participates in the pathogenesis of puerperal sepsis [7].

2.2.6 Characterisation and classification of strains

The classical serological typing schemes for GAS are based on the variability of antigenic surface-exposed proteins, such as the T protein, the serum opacity factor protein, and the M protein. T serotyping, based on agglutination of the T antigen with type-specific sera, identifies approximately 25 distinct T types. It was introduced in 1965 and has been widely used for the characterisation of strains [224]. A characteristic of T typing is that it results in complex agglutination patterns due to strains harbouring several T protein antigens [156, 159]. The function of the T protein as a pilus-like structure, contributing to the virulence of the bacterium, was discovered only recently [225]. Sequence variation of the pilus backbone variant, *tee* sequence typing, has been proposed as a molecular typing tool to substitute for the serological T typing [104]. Detection of the presence of the SOF protein and its specific type has also been used for typing purposes of GAS and it has provided a useful tool for initial screening and characterisation of GAS, especially when combined with T typing [207].

Serologic M typing, originally introduced by Dr. Rebecca Lancefield, is based on variation in the streptococcal M protein [187]. With this method, still in use in some countries, GAS can be classified into 80 serotypes by the difference in the hypervariable N-terminus of the M protein (see also Figure 1 in section 2.2.4). Because specific M, T, and OF types correlate with each other and some type combinations have been associated with certain clinical manifestations and severity of disease, these methods have been used in unison to obtain more specific information on the diversity of strains [19, 72, 99, 156, 159]. Common problems encountered with serological typing methods are limitations in the specificity and availability of typing antisera, leading to ambiguous results and a large number of

nontypable isolates. Conventional methods are therefore being replaced with, or strengthened by, the use of molecular methods.

The first alternatives to serological M typing were PCR-based methods with probes directed to recognise different types of M-protein in a method called M-ELISA typing, based on an enzyme-linked immunosorbent assay [174, 250, 252, 267]. Recently, *emm* (sequence) typing, based on sequencing a 180 bp portion of the hypervariable 5' terminus of the *emm* gene, has become the 'gold standard' method for genotyping of streptococci [17, 88, 100]. In addition to the designation of *emm* types to the the original serologic M types up to M81, new *emm* types of 82-124 have been validated and added accordingly [100, 101]. At present, well over a hundred *emm* sequence types and a far higher number of subtypes have been identified and stored at the *Streptococcus pyogenes emm* sequence database at the Centers for Disease Control and Prevention (CDC) [45, 101]. The *emm* typing results are accurate, unambiguous, and easily comparable. *emm* typing provides good discriminatory power of isolates, but for the purpose of investigating the clonality of strains, such as in outbreak investigations, *emm* typing is best when complemented by other methods, serological or molecular [18, 19].

Pulsed-field gel electrophoresis (PFGE) targets the whole genome of the bacterium and is a widely used typing method for investigating genetic relationships between isolates for many bacterial species [283, 313]. The whole bacterial DNA is digested with a rare-cutting restriction enzyme (usually SmaI for GAS) to obtain a relatively small amount of fragments 20-800 kb in size, which are separated in a specific gel electrophoresis apparatus that periodically switches the direction of the electrical current, allowing the separation of large DNA fragments. Random genetic events such as deletions, insertions, and point mutations sometimes alter the restriction sites and affect the restriction pattern of a strain. PFGE has a high discriminatory power that can be enhanced by the choice of an appropriate cutting enzyme, and it is most useful in epidemiological studies and outbreak investigations. The downside of this method is that it is time-consuming and interlaboratory comparison of strain patterns is difficult.

Multilocus sequence typing (MLST) is based on sequencing seven highly conservative "housekeeping" genes necessary for cell functions and it results in distinct allelic profiles called sequence types (ST). The STs are highly concordant with other typing methods such as *emm* typing, but MLST can usually discriminate two or more STs among isolates of a single *emm* type [44, 93]. MLST is considerably expensive and laborious, but its advantages are easy comparison of allelic profiles between laboratories and the unambiguous numerical form of data. An MLST database for several bacterial species is available in the internet [222].

When performed coupled with *emm* typing, either MLST or PFGE provide more discriminatory power and benefit to clonal analyses than is possible to obtain by *emm* typing alone [44].

Other genomic typing methods that have been used for characterisation of GAS are restriction endonuclease analysis (REA) typing and fluorescent amplified-fragment length polymorphism analysis [78, 79, 274]. Some typing methods are restricted to specific genomic areas or genes. Vir typing was developed before *emm* typing; it is a restriction fragment length polymorphism (RFLP) analysis of the pathogenicity island encoding *emm* and other virulence genes [113]. Sequence analysis of the hypervariable *sic* gene coding for the streptococcal inhibitor of complement protein has also been used for clonal analysis of M1 isolates in more detail. This method is very effective in discriminating distinct clones, as the *sic* gene polymorphism is extensive, but its use is naturally restricted to strains harbouring the *sic* gene [136, 297]. Ribotyping is based on the analysis of polymorphisms of 16S rRNA genes and has been used to characterise bacterial isolates of many species, but may lack discriminatory power compared to MLST [81, 290].

The oligonucleotide microarray is a novel technology that has been used for efficient and accurate detection of GAS with the additional benefit of identifying erythromycin resistance markers [76]. This technology offers promising possibilities for diagnostic and genotyping purposes, as it could be expanded to cover a wide array of genes, such as additional antimicrobial resistance genes, the *emm* gene, and SAg genes.

2.3 The epidemiology of *S. pyogenes* infections

Throughout the 20th century, the prevalence of rheumatic fever and other severe infections by GAS declined dramatically in developed countries [167, 206]. One reason for this decline was the development and availability of antimicrobial agents [77]. Other probable reasons include the improved socio-economic conditions, application of aseptic techniques, and a decreased prevalence of virulent strains [115, 173]. For several decades until the mid-1980s, the morbidity and mortality due to GAS infections and their sequelae was quite low and consequently the GAS infections were not often considered to be serious [88, 157].

However, during the latter half of 1980s and continuing into the 1990s, a change in the epidemiology of invasive group A streptococcal infections, with an increase in the incidence as well as severity of disease, was documented in many developed countries [42, 88, 115, 140, 157, 270, 295]. Cases presented with a range of diseases including bacteraemia, necrotizing fasciitis and toxic shock [53, 295]. A high

prevalence of strains producing SpeA toxin was noted [53, 295]. Simultaneously, the incidence of acute rheumatic fever increased in the USA, although this was later discovered to be only a temporary increase with ARF later practically disappearing in the USA [167, 278, 326, 327]. Indications of an increasing incidence of erysipelas infections with the predominant site changing from the face towards the leg were also observed [48, 264]. The role and pathogenic potential of type M1, M3 and M18 in the epidemics was of specific interest, as these types were disproportionately associated with invasive infections [53, 59, 157, 204, 270, 300]. Type M18 was also found to be associated with acute rheumatic fever [157, 270].

The reasons for the resurgence of the invasive infections are still not fully understood. This could have resulted from a change in the pathogen or in the protective immunity of the population. A hypothesis prevailed that the emergence of highly virulent clones of GAS was the primary explanation behind the surge of invasive infections, but no single clone was found to significantly associate with invasive infections [158, 263]. A possible role of the population's lower level of immunity towards the invasive types was suggested, but it was unlikely to be the leading cause for the resurgence [53, 270]. An event likely to contribute was a shift in the prevalence of specific types or strains among GAS with certain virulence factors leading to increasing numbers and widespread transmission of these strains among the general population at the time [53, 157, 263]. Recent studies point to horizontal gene transfer events leading to the evolution of a new, unusually virulent clone of type M1 that increased dramatically in frequency since the 1980s [301].

Virulent clones causing invasive disease are indistinguishable from strains causing uncomplicated pharyngitis [49, 157, 158, 228]. It is highly probable that the majority of patients developing invasive infections are infected through contact with individuals, most likely children, with relatively benign infections such as pharyngitis, as direct transmission of invasive disease is rare [73, 96, 158]. Thus the importance of pharyngitis and pyoderma in leading to not only post-streptococcal disease, but also secondary cases of invasive disease, cannot be ignored [43, 125, 128, 137, 205, 228]. The fact that infections by the same clone of GAS can manifest themselves differently depending on the individual, also point to a strong role of host factors influencing the outcome of an infection (see also section 2.5) [49].

Although the risk of a subsequent iGAS case after transmission of the pathogen from the patient to another person is higher than the risk of iGAS disease among the general population, the risk is considered low and routinely administered chemoprophylaxis is not recommended [262, 286]. The suggested alternative action is to inform all household contacts of the clinical manifestations of invasive disease and to advise them to seek immediate medical attention if such symptoms arise [286].

2.3.1 Estimates of overall disease incidence

Severe *S. pyogenes* infections are not notifiable diseases in many countries. The methods, criteria, and coverage of severe GAS disease surveillance vary considerably by country and may depend on voluntary reporting systems [182]. Some countries include only bacteraemia and cerebrospinal fluid (CSF) isolations whereas in some countries the case definition of severe iGAS disease is wider, including also isolations from other normally sterile sites and STSS cases without bacteraemia. For this reason, the comparison of disease rates between countries is challenging and relatively few population-based studies with results from several consecutive years exist.

The Strep-EURO-network was set up in 2002 and aimed at improving knowledge of the epidemiology of iGAS and unifying the surveillance strategies used for severe GAS disease in Europe [182, 185]. This collaboration involved collection of epidemiological, clinical, and microbiological data. Since the resurgence of GAS infections, there has been a general view of an increasing incidence of invasive disease during the 1990s and 2000s in many European countries [182]. In the Strep-EURO study, Finland, Denmark, Sweden, and the UK had the highest rates of infection (range 2.5-3.3 cases per 100,000) during 2003-2004 in Europe [185].

The European findings are somewhat in contrast to the situation outside Europe. The incidence in the USA has remained relatively stably at an average rate of 3.5 cases per 100,000 during 1995-2004 [242, 244]. The statistics from the Active Bacterial Core Surveillance show a moderate increase recently, with a rate of approximately 3.8 per 100,000 in 2007 [46]. Studies from Canada showed a steady rate in the range of 2.4-3.3 cases per 100,000 during 1998-2001 in Montreal but higher rates of 4.3 during 1999-2004 in Calgary and 3.8-5.7 during 2000-2002 in Alberta [143, 188, 322]. In temperate Australia, Victoria, a rate of 2.7 cases per 100,000 during 2002-2004 was reported [243].

The incidence rates in developed countries within and outside Europe are at a relatively similar level. The fluctuation in incidence is characteristic for this disease, as variations and occasional peaks occur over time and within defined geographical regions [185, 244, 322]. This is possibly a reflection of the susceptibility of a given population to particular strains with diverse virulence properties. Nonetheless, the estimates of incidence of iGAS disease in developing countries such as Fiji and Kenya and among the indigenous populations within developed countries such as Australia and New Zealand have shown considerably higher rates of infection [25, 140, 241, 291].

GAS is an important cause of morbidity and mortality, mainly in less developed countries [43]. The global disease burden of severe GAS diseases is considerable: a report by the WHO estimated that currently approximately 18.1 million people experience the consequences of a severe GAS infection, with 1.78 million new cases occurring each year [43]. Among these, at least 663,000 new cases of invasive disease and 163,000 deaths occur each year [43]. In reality, the true disease burden could be much greater.

2.3.2 Age- and sex-specific rates of infection

There are certain characteristics in the age-specific rates of infection that are commonly observed. Children under 5 years of age and the elderly (≥65 years) generally have a higher incidence of iGAS disease compared to other age groups [72, 185, 198, 322]. The incidence rate in children aged <5 years has been at a level of 2 or less cases per 100,000 in Scandinavian countries of Sweden and Denmark [72, 198], which is lower than is observed in the UK, the USA, Canada and Australia [185, 243, 244, 322]. A slight male dominance in iGAS infections has been noticed in several studies [185, 188, 243, 244], but also some contrasting findings have been published [72, 90].

2.3.3 Mortality associated with invasive infections

The WHO estimate of 163,000 deaths occurring globally each year due to iGAS infections is likely to be an underestimate, because data from many developing countries are unavailable [43]. The overall case fatality rates due to iGAS infections as reported by countries within and outside the Europe vary in the range of 7-25% [72, 90, 184, 198, 243, 244, 322]. The case fatality is dependent on the streptococcal M type (especially types 1 and 3 have been associated with higher case fatality), age of the patient (the rate increasing with age), and whether the infection is associated with necrotizing fasciitis (higher case fatality of 17-45%) or if the patient develops STSS (23-81%, respectively) [72, 73, 90, 96, 185, 196, 241, 242]. Residents of long-term care facilities have a higher incidence and case fatality rates due to iGAS infections than community-based case-patients [315].

Differences in the case fatality rates are also subject to what information is available. The case fatality rate can be presented as deaths within 7 or 30 days after the onset of infection, or outcome at the time of hospital discharge, or some other measure. Further, the rates are subject to bias in the reporting system, since with non-automatic reporting systems and the coverage of surveillance being less than 100%, it is possible that the more severe cases are more likely to be reported than the milder cases.

2.3.4 Clinical presentations

There are quite a few recent epidemiological studies published in Europe (Sweden, Denmark, the Netherlands, the UK) and outside Europe (the USA, Canada and Australia), including detailed information of clinical presentations of iGAS disease [72, 90, 185, 184, 198, 242-244, 300, 322, 333]. The individual studies differ somewhat from each other in the criteria used for defining some of the clinical presentations. Many patients may also have more than one clinical presentation, i.e. the data are overlapping. The ranges of percentages for each clinical presentation presented next are compiled from these studies.

The most common manifestations of severe *S. pyogenes* infections are skin and soft tissue infections (including most importantly cellulitis, but may also include necrotizing fasciitis, abscess, and erysipelas). These infections usually account for a range of 20-40% of the cases but in some studies more than 50%, depending on how broad the definition of skin and soft tissue infections is, but also on geographical differences in the epidemiology of these infections. However, quite commonly, 12-30% of the patients with a severe *S. pyogenes* infection have bacteraemia with no focal symptoms or source. Other commonly reported individual clinical presentations in the order of prevalence are pneumonia (10-15%), septic arthritis (5-15%), necrotizing fasciitis (5-13%), STSS (6-11%), puerperal sepsis (2-7%), and meningitis (1-4%). Most iGAS cases arise in the community; according to some epidemiological studies, 4-13% of iGAS infections are healthcare-associated (hospital-acquired) [71, 182, 185].

2.3.5 Trends in *emm* type prevalence

Certain *emm* types feature regularly among the most prevalent types causing invasive infections in developed countries, in particular 1, 3, 12, 28, and 89 [72, 90, 135, 195, 200, 198, 243, 244, 323]. The five most prevalent types may account for approximately 50% or more of the total amount of isolates [198, 242, 244]. Table 4 lists a comparison of epidemiologic studies of iGAS with *emm* type information published in the 2000s. The results of these and earlier studies point out that the prevalence of specific GAS types, both in mild and severe infections, shows high variability over time and geographical area [72, 168, 242, 268, 280]. In any given population, a continuous flux of common and sometimes uncommon *emm* types seem to emerge and replace the prevalent types. Thus, the prevalence of certain types in invasive or noninvasive infections is a reflection of the circulating types of *S. pyogenes* in the general population at a given time.

There are some studies of the *emm* type distribution in developing countries and countries from which there is no previous information, such as Ethiopia, India, Fiji, and Brazil, where the diversity of types may be larger and the type distribution different compared to that of European countries or the USA [2, 80, 266, 291, 312, 314].

Table 4. Comparison of epidemiologic studies of invasive S. pyogenes disease and prevalent emm types in the 2000s.

Country	Reference	Time of study	No. isolates (no. *emm* typed)	Incidence per 100,000 population	Case fatality rate (%)	*emm* type (%)										
						1	**3**	**4**	**11**	**12**	**28**	**77**	**81**	**89**	**91**	**PT2967**
Alberta, Canada	[322]	2000-2002	441 (433)	4.8[a]	10.6[b]	16	12	4	5	7	5	-[c]	-	-	5	10
USA	[244]	2000-2004	5400 (4350)	3.5	13.7[d]	22	9	-	-	9	9	-	-	6	-	-
Denmark	[90]	2001-2002	201	-	25[e]	32	4	5	-	6	20	3	-	10	-	-
Sweden	[72]	2002-2004	746	3.0	14.5[b]	12	-	6	-	6	14	6	15	16	-	-
Victoria, Australia	[243]	2002-2004	333 (255)	2.7	7.8[b]	26[a]	-	-	-	-	10[a]	-	-	-	-	-
Denmark	[198]	2003-2004	278	2.6	16[f]/20[e]	24	11	-	-	6	26	-	-	7	-	-

[a] estimate based on data given in the publication

[b] time not specified

[c] -: data not available

[d] outcome at the time of hospital discharge

[e] 30d case fatality

[f] 7d case fatality

2.3.6 Association of *emm* type and disease manifestation

Epidemiological studies have shown the existence of non-random associations of M types to particular infections [115, 195, 242, 323]. Types M1 and M3 are particularly associated with invasive and fatal infections (and also with STSS) but are also common in pharyngeal infections [59, 72, 157]. Similarly, M28 is commonly found among invasive and pharyngeal infections, but also significantly overrepresented among puerperal sepsis and neonatal GAS infections [51, 59, 72, 98, 121]. In addition to the aforementioned types, especially type M12 has been encountered as the most prevalent type among non-invasive pharyngeal isolates but it is not uncommon in invasive disease either [72, 157, 278, 280, 309].

The so-called rheumatic M types particularly associated with acute rheumatic fever are 1, 3, 5, 6, 18, 19, and 24; also types 14, 27, and 29 have been known to cause ARF [59, 157, 278, 298]. The most important M serotypes associated with acute poststreptococcal glomerulonephritis (the nephritogenic types) are 49, 57, and 60 [59, 157, 217]. M80 and M81 have been known to be more common in pyoderma and are more frequently encountered in infections that involve the skin and tissues [59, 72].

Isolates of a single *emm* type may represent a wide array of distinct strains, which may greatly differ in their genetics, virulence potential or epidemiological characteristics [211]. Indeed, the genetic diversity of adhesive molecules may reflect the types of diseases caused by GAS [21]. It is also important to keep in mind that the associations between the *emm* type and clinical and epidemiological properties of GAS that are observed in one geographic region are not necessarily applicable in other parts of the world [211].

2.3.7 Factors predisposing to invasive *S. pyogenes* infections

Several host and environmental factors have been identified as predisposing factors to *S. pyogenes* infections, and they have a potential role in the severity and outcome of infection [73]. Age is an important host factor and certain age groups are at a higher risk of developing invasive disease, such as small children and especially the elderly. For both adults and children, household overcrowding and exposure to children with sore throat have been found to increase the risk of infection [103]. Common risk factors for adults also include underlying diseases such as cardiac disease, cancer (with local radiation therapy causing tissue damage), and diabetes [73, 102, 198, 242-244, 304]. Specific risk behaviours, such as alcoholism and injecting drug use, are also associated with the increased risk of invasive infection [73, 102, 184, 242, 244]. Skin conditions (burns, trauma, and surgical wounds) understandably predispose to infections [244]. A viral infection, such as a varicella-

zoster virus (causing chickenpox) or HIV, has also been identified as a risk factor [73, 102, 189, 243]. Institutional acquisition (iGAS aquired in a hospital, nursing home or long-term care facility) is a common risk factor [198, 315, 322]. Yet, approximately 17-20% of patients with iGAS infection have no predisposing factors [185, 242].

2.3.8 Seasonal variation

One feature of *S. pyogenes* infections as identified in many countries is a seasonal variation in the number of cases. In Europe, such as the UK, Sweden, and the Netherlands, as well as in the USA and Canada, a peak of invasive cases has been observed in winter/spring months, with a lower incidence in late summer/autumn [72, 73, 185, 244, 322, 334]. Similar to invasive infections caused by *S. pyogenes*, streptococcal pharyngitis shows a seasonal pattern towards the autumn and winter, whereas skin related infections have been found to be more prevalent during the summer [64, 91, 223, 264].

The reasons for seasonal variation are not clear but several factors may have a role: environmental changes (humidity or temperature) affecting the mucosal defence barriers, behavioural patterns such as crowding of susceptible persons as a result of cold weather or school seasons, changes in susceptibility of the human host associated to the annual light/dark cycle, or viral respiratory infections inducing vulnerability to *S. pyogenes* infection [82, 83, 185]. Increased exposure of skin to superficial trauma during the summer, as well as a delayed wound healing, may contribute to the seasonal variation for skin and soft tissue infections such as cellulitis [264].

2.4 Preventive measures and treatment

2.4.1 Antimicrobial resistance and treatment strategies

Antimicrobial resistance is a common problem worldwide and the consumption of antimicrobials has been identified as a driving force for the emergence and transmission of antimicrobial resistance in bacteria. Group A streptococci are sensitive to penicillin and other β-lactam antimicrobials, such as cephalosporins and carbapenems [294]. Penicillin is therefore the first drug of choice for treating pharyngeal and other streptococcal infections [171]. The continuing susceptibility of *S. pyogenes* to penicillin is remarkable and possibly due to a limited ability to aquire foreign DNA and a lower fitness that may be associated with β-lactam resistance [146]. Resistance to sulfonamides and tetracyclines in group A streptococci has been decscribed already in the mid-1960s [294]. The development of antimicrobial

resistance among other bacterial species warrants continuous monitoring to guide the appropriate antimicrobial therapy and vaccine development strategies [260].

The potential for increased resistance to erythromycin and other macrolides, as a consequence of the widespread use of macrolide antimicrobials, has been widely studied [276, 294]. There are two main mechanisms of erythromycin resistance in GAS. Firstly, the resistance can be acquired by target site modification; a methylase encoded by one of several types of *erm* (erythromycin resistance methylase) genes causes a conformational change in the bacterial ribosome, which is the target of erythromycin [192]. This kind of resistance can be constitutive (cMLS phenotype; *ermB* gene) with a continuous production of the methylase enzyme, or inducible (iMLS phenotype; *ermA* gene) when the production of the enzyme occurs only in the presence of an inducing antimicrobial [273]. A newly discovered *ermTR* resistance methylase gene was found to be predominant in Finnish *S. pyogenes* isolates and was found mainly in isolates with the inducible phenotype [170]. A second resistance mechanism is based on active macrolide efflux by the presence of a membrane-associated pump mechanism encoded by the *mefA* gene (the M phenotype) [193]. The constitutive phenotype is manifested in double-disk diffusion tests by resistance to both erythromycin and clindamycin, while the inducible phenotype is shown by erythromycin resistance and lowered susceptibility to clindamycin induced by the proximity of erythromycin disk. Isolates with the M phenotype are erythromycin resistant and clindamycin susceptible [170].

High or increasing prevalence of macrolide resistant *S. pyogenes* isolates has been identified in many countries, such as Italy, Spain, Portugal, France, and Japan, and lately in Asian countries, such as China, Taiwan, and South Korea [6, 29, 63, 119, 155, 166, 208, 220, 281, 324, 325]. Spain and Germany, among other countries, have had a few prevalent erythromycin-resistant clones which have been the main reason for increased resistance [6, 251, 259]. In France, the emergence of clonally related multiresistant *emm*28 isolates was noted in the early 2000s [221]. In Finland, an increase of erythromycin resistance of *S. pyogenes* with new resistance phenotype was recognised in the early 1990s, but after restrictions in the use of macrolides, the resistance steadily decreased [24, 172, 275, 276, 272]. Countries such as Australia, the USA, and Denmark have recently seen a reasonably low level of macrolide resistance with moderate or no increase of resistant strains [198, 243, 260]. Contrastingly, Romania, Israel, and Iran have had a high rate of tetracycline resistant strains with an absence of erythromycin resistance [154, 199, 261, 328].

In Finland, the recommended antimicrobial treatment for streptococcal pharyngitis as the first choice is oral penicillin V three times a day for 10 days, and for patients with penicillin allergy, a first generation cephalosporin (such as cephalexin) as the

second choice [86]. The recommended antimicrobial treatment for streptococcal cellulitis is penicillin administered either intravenously or intramuscularly, and after the recovery has started, usually after 3-5 days, perorally for a total of three weeks [85]. For patients who are penicillin-allergic, cephalosporins are recommended, or, alternatively, clindamycin in cases with severe penicillin allergy. In patients with blood culture positive GAS finding, the first-line antimicrobial agent is either intravenous penicillin G or a cephalosporin [84].

Pooled human immunoglobulin G (IVIG) and polyspecific immunoglobulin IgM, IgA, and IgG preparations may have beneficial effects in the treatment of STSS [32]. The therapeutic mechanism may at least partly arise through the increasing level of M1-specific antibodies, which help in the elimination of bacteria and in toxin neutralisation [14, 235, 238, 237]. Despite availability of treatment, severe infections complicated by STSS are associated with a high case fatality rate.

2.4.2 Vaccine candidates

The search for a vaccine to prevent GAS infections has been ongoing for decades and at present, several candidate GAS vaccines are under research and development. As mild infections sometimes lead to more severe infections, a vaccine that would protect against pharyngeal infections could also theoretically at least partly protect against more severe infections [128]. Untreated streptococcal infections leading to sequelae are a problem especially in developing countries, which could benefit from the use of a vaccine to prevent the primary infections. Bacterial structures that are associated with adhesion to host cells, such as lipoteichoic acid, fibronectin binding proteins, M protein, and T protein, are natural targets for vaccine development.

The M protein, being a major virulence factor of GAS, has been of specific interest in vaccine design [187]. Opsonic antibodies directed against the N-terminal part of the M protein are mostly responsible for serotypic immunity, but the exceeding number of existing M types poses a problem. Nonetheless, according to epidemiological studies made in the USA and elsewhere, the majority of GAS infections are caused by a relatively small number of M types [56, 185, 243, 244]. Thus, a multivalent M-protein based vaccine including the most prevalent types could prevent a considerable proportion of GAS infections within a defined population and have a significant impact on the burden of streptococcal diseases [67, 216]. However, the observation that some epitopes of M proteins can induce antibodies that cross-react with human tissue and potentially induce autoimmunity has hindered attempts to develop an M protein-based vaccine [10, 40, 68, 69, 245]. A safe and effective vaccine must be capable of inducing broad strain-protective antibodies against GAS and clearing the infection without triggering autoimmune

responses [245]. Development of several M protein-based vaccines containing only the protective epitopes but with no tissue cross-reactive epitopes is underway.

Among the multivalent M protein-based vaccines, the first one to go into phase I clinical trials was a hexavalent recombinant fusion protein peptide containing N-terminal M protein fragments from serotypes 1, 3, 5, 6, 19, and 24 [66, 179]. The second generation M protein-based recombinant vaccine contained 26 M types and a newly found streptococcal protective antigen (Spa) [67, 70, 147, 216]. The vaccine includes epitopes from M types 1.0, 1.2, 2, 3, 5, 6, 11, 12, 14, 18, 19, 22, 24, 28, 29, 33, 43, 59, 75, 76, 77, 89, 92, 94, 101, and 114. These M types have been selected on the basis of being epidemiologically significant; they are commonly responsible for serious infections or uncomplicated pharyngitis in children or are associated with ARF [216]. The 26-valent vaccine candidate has undergone phase I and II clinical trials with promising results which warrant studies in adolescents and children, who are the target population for this vaccine [67, 216, 215]. The downside of this vaccine candidate is that it may not be effective in preventing infections within other populations such as developing countries, where the diversity of GAS M types may be very different [2, 80]. The vaccine may also need re-evaluating and redesigning to accommodate future changes in type prevalence among the original target population.

A different approach is a vaccine containing epitopes from the more conserved C-repeat region of M protein [16, 285, 335]. This kind of vaccine would potentially protect against a broader spectrum of GAS *emm* types and its coverage would not be dependent on the M type distribution of a given geographical area. Synthetic multi-epitope vaccines combining N- and C- terminal epitopes are also being investigated [39].

Selection of the appropriate adjuvant and carrier systems are essential for effective vaccine delivery. Several carrier molecules have been considered for conjugates of vaccine antigens, such as synthetic carbohydrates, diphtheria toxoid protein, and glycolipids attached to peptide antigens to form lipid-core peptides, the latter of which seem particularly promising for possible mucosal administration [16, 112, 246, 282]. Mucosal vaccines are easy to administer and offer advantages over classical parenteral immunisation, with the possibility to induce both systemic and mucosal immune responses and thus provide enhanced protection against pharyngeal GAS infections [245]. One could expect a significant benefit from a mucosal immune response in the prevention of pharyngitis, as mucosal IgA would have the potential to attach to pathogens and prevent them from adhering to the mucosal epithelium [303, 321].

2.5 Susceptibility to streptococcal infections

Despite the extensive studies aimed at understanding the virulence properties of streptococci, the key question remains: Why do some individuals develop a severe disease while others only suffer a mild pharyngitis? Human genetic variation is a key element in infectious disease susceptibility, as it determines the quality and strength of individual immunological responses that have a role in the severity and outcome of infections [132]. It is not surprising that the most numerous and diverse genes in the human genome are indeed genes involved in the immune response, having evolved under selective pressure by infectious agents [111, 229]. Specific genes that have a role in the innate immunity, such as the family of pathogen pattern recognizing receptors and their associated signalling pathways, have been subject to most extensive research in this aspect. Evidence of host genetic factors playing a role in the susceptibility to infectious diseases has already been discovered for malaria, tuberculosis, and invasive pneumococcal disease, to name a few [36, 132]. Based on these and other studies, it is most likely that the genetic basis of susceptibility to streptococcal diseases is not monogenic (due to one gene only) but polygenic with many minor contributory loci [111, 132]. Given the large number of factors contributing to host defence against GAS infections, identification of individual genes remains a difficult challenge.

The lack of protective neutralizing antibodies against the M proteins and possibly to other virulence factors seems to be a risk factor for developing severe disease [15, 97, 144, 292]. Furthermore, the lack of protective anti-SAg antibodies has been found to be associated with the increased risk for developing STSS [15, 97]. However, the quality of the protective anti-Spe antibodies (as measured by the neutralizing activity), rather than the quantity of them, is a more important determinant in disease pathogenesis [15]. Thus, previous contact with the pathogen would help to protect from severe disease, but in case the infection progresses to bacteraemia, the course of infection would only partly be determined by host levels of antibody to SpeA but also by patient's age, underlying diseases, and possible immunocompromisation [15, 292].

The magnitude of inflammatory responses triggered by superantigens also varies considerably by individual. A direct correlation has been found between the intensity of inflammatory cytokine response and the severity of the infection [236]. Thus variations in genes regulating the inflammatory response are of interest. The human leukocyte antigen (HLA) class II molecules are receptors for SAgs. Indeed, an association between specific HLA class II haplotypes and the outcome of invasive GAS infection has been identified, as certain haplotypes conferred protection from severe systemic disease by attenuated inflammatory responses to SAgs [178].

Autoimmunity is also linked to the susceptibility to infection. Increasing evidence exists of the role of an autoimmune process in the pathogenesis of acute rheumatic fever [124]. The epidemiological data supporting this hypothesis indicate differences in familial and racial incidence, with a markedy higher attack rate in individuals who have had a previous episode of ARF [30, 52, 202]. Even more importantly, there is immunological evidence of ARF patients having hyperimmune responses to streptococcal antigens, and that this is associated with certain HLA class II alleles [8, 123, 255, 279].

Research on genetic susceptibility to infectious diseases involves genetic studies of both the host and the pathogen, and of the functional interactions between the two. There are alternatives to studying the genetics of the host, such as linkage and association analyses. Large scale genome scans with several hundreds of markers (e.g. microsatellites or single nuclear polymorphisms, SNPs) across the genome are now routinely performed to obtain data for these analyses. Linkage analyses aim to identify the link between certain markers and the presence of the disease in families and to pinpoint certain genomic regions of interest. In studies of familial aggregation of infectious diseases, it is most important that the disease phenotype is carefully defined and its diagnosis is straightforward. Furthermore, the effect of possible transmission of the pathogen between the family members has to be taken into account. Association analyses compare the allele frequencies of candidate genes between those with the disease and control subjects and aim to determine if there is statistical difference in the gene frequencies.

Because *S. pyogenes* is strictly a human pathogen, there are limitations of animal models of GAS disease. Despite this obstacle, laboratory mice can be experimentally infected with GAS and several murine models of infection have been developed to study the pathogenesis of GAS infections and the inflammatory reactions in mice [218]. However, results from animal models of disease may not be directly applicable to research of human susceptibility.

Genetic studies of the pathogen involve characterising the pathogen with methods of choice and looking for association between the disease phenotype and the properties of the pathogen [36]. Both human and bacterial genetic research has greatly benefited from the availability of rapid and more efficient SNP genotyping methods and DNA microarrays [38, 132]. Microarray-based gene expression analysis has also been used for analysing changes in gene expression of host cells during GAS infection [117].

3 AIMS OF THE STUDY

The purpose of this study was to investigate the recent trends in the epidemiology of invasive *Streptococcus pyogenes* infections, molecular properties of invasive isolates, and the microbial aetiology of acute bacterial non-necrotizing cellulitis infections in Finland.

The specific objectives of this study were:

1. To analyse and compare the T serotype distribution of invasive *S. pyogenes* isolates in Finland to other countries and to further investigate the molecular epidemiology and clonality of serotype T28 through *emm* typing (I).
2. To investigate the molecular properties of isolates of type *emm*84, a new and uncommon type in Finland, and to compare *emm*84 cases in terms of geographical, patient and outcome information to other cases of invasive disease (II).
3. To analyse and compare the epidemiology of invasive *S. pyogenes* infections in Finland to other countries according overall, annual, age- and sex-specific incidence rates, seasonal patterns of infection, *emm* type distribution, antimicrobial susceptibility, and overall and type-specific case fatality rates (III).
4. To isolate and characterise β-haemolytic streptococci causing non-necrotizing cellulitis infections with geno- and serotyping methods through a population-based case-control study, compare streptococcal isolates to those causing invasive disease and evaluate pharyngeal carriage of β-haemolytic streptococci in cases, their household members and control subjects (IV).
5. To assess the usability and discriminatory power of different bacterial typing methods for determining the clonality of strains for epidemiological surveillance and outbreak investigations.

4 MATERIALS AND METHODS

4.1 Invasive *S. pyogenes* disease

4.1.1 Surveillance

In Finland, with a population of 5.3 million, the national healthcare system is organised into 20 healthcare districts, with catchment populations ranging from 58,000 to 1.5 million. The healthcare districts form five tertiary care districts, each with a University Hospital in a major city. Since 1995, all clinical microbiology laboratories across Finland have mandatorily notified all *S. pyogenes* (group A streptococcus) isolations from blood or cerebrospinal fluid (CSF) to the National Infectious Disease Register (NIDR) held by the National Institute for Health and Welfare, THL (formerly the National Public Health Institute of Finland) [316]. With each notification, the following information regarding the isolation and patient is transmitted (generally electronically) to NIDR: date and type of specimen, unique national identity code of the patient (since 2004), date of birth, sex, and place of treatment. Notifications concerning the same patient within a time interval of three months are merged into a single case. The GAS isolates corresponding to the notifications are submitted to the national reference laboratory at THL for confirmation, typing, and culture collection.

4.1.2 Case definition and outcome

A case of invasive GAS was defined as *S. pyogenes* isolated from blood or CSF. This study included iGAS cases and the corresponding isolates from January 1995 to December 2007. Information on the vital status of the case-patients at 7 days of positive blood or CSF culture was obtained for 2004-2007 from the Population Information System held by the Population Register Centre through the use of the national identity codes.

4.1.3 Collection, culturing and identification of isolates

Blood and CSF samples were processed by the clinical laboratories of the healthcare districts. Isolates of *S. pyogenes* from all culture positive specimens were sent to THL in swab culture tubes. The bacterial isolates were cultured on sheep blood agar and incubated in 5% CO_2 at 35°C, and bacterial growth was determined at 24 h and 48 h. Isolates were confirmed as *S. pyogenes* by β-haemolysis on blood agar, sensitivity to bacitracin, and detection of the Lancefield group A antigen by Streptex latex agglutination test (Remel Europe Ltd). The isolates were stored for culture collection at -70°C.

4.1.4 Review of patient medical records

A cluster of invasive infections by type *emm*84 occurring within one hospital in one healthcare district was investigated in more detail (publication II). Patient medical records were reviewed in order to identify underlying conditions and if the patients had had any common exposure or contact with each other.

4.2 Acute streptococcal non-necrotizing cellulitis

4.2.1 Study design and population

The case-control study of acute streptococcal non-necrotizing cellulitis targeted individuals presenting with acute cellulitis and erysipelas infections during April 2004-March 2005. Patients ≥18 years of age hospitalised for acute cellulitis or erysipelas infections were recruited into the study from two infectious diseases wards in two hospitals in the City of Tampere (catchment population 500,000). Patients potentially meeting the case definition were assessed and the diagnosis confirmed within 4 days of admission to the hospital by an infectious diseases specialist. For each patient, one control subject matched for age, sex, and residence was recruited by randomly sampling candidates from the Population Register Centre. Where possible, family members sharing the same household as the patients were also recruited.

4.2.2 Case definition and exclusion criteria

Acute, bacterial non-necrotizing cellulitis was defined as either of the following: (1) a sudden onset of fever or chills and a localised, more-or-less well-demarcated erythema of the skin in the leg or arm or, (2) a typical appearance of well-demarcated skin eruption on the face, either with or without fever or chills. Thus, bacterial cellulitis and erysipelas (as the superficial form of cellulitis) were included in the case definition. Patients who had cutaneous abscesses, NF, septic arthritis or osteomyelitis were excluded from the study.

4.2.3 Collection of samples

Samples were collected from the recruited patients using sterile culture and transportation tubes (Technical Service Consultants). Skin swab samples were taken from any existing wound or blister within the infection focus or if the skin area was intact, elsewhere from any abrasion or fissured toe web. A throat swab sample was taken from all patients and control subjects and where available from household members. Blood samples were drawn from all patients for determination of bacteraemia.

4.2.4 Culturing and identification of samples

All skin and throat swabs were sent to THL. The swab was first used to inoculate a sheep blood agar plate and then placed in a tube of sterile water in order to obtain a bacterial suspension. The suspension was serially diluted and an aliquot of the dilutions were plated on sheep blood agar. All plates were incubated in 5% CO_2 at 35°C, and bacterial growth was determined at 24 h and 48 h. β-haemolytic bacterial growth was visually examined and recorded and the number of colony forming units/ml calculated. From each sample, up to 10 suspected β-haemolytic streptococcal colonies and one suspected *S. aureus* colony were picked for further culturing and identification.

The processing and identification of blood cultures from patients was performed by the clinical laboratory of the respective hospitals using standard culture systems and media (Bactec 9240, BD Diagnostic Systems). Any β-haemolytic streptococcal isolates were forwarded to THL on blood agar plates.

All suspected β-haemolytic streptococcal isolates were tested for sensitivity to bacitracin and the Streptex latex agglutination test (Remel Europe Ltd) applied to detect the Lancefield group antigens A, B, C, D, F, and G. The Staph Slidex Plus latex agglutination test (bioMérieux) was used for identification of *S. aureus*. The species of group A, B, and G streptococci were determined by API ID 32 Strep test (bioMérieux). Isolates positively identified as β-haemolytic streptococci or *S. aureus* were stored at -70°C.

4.3 Characterisation of isolates

The methods applied to characterise the invasive *S. pyogenes* isolates and isolates from the case-control study of cellulitis in the different publications of this work is described in detail in Table 5.

The invasive isolates were analysed according to the typing methods available in the reference laboratory at the time the isolate was received. T serotyping was used for characterisation of all isolates during 1995-2006. *emm* typing was started in 2003 and used as the primary typing method for all iGAS isolates during 2004-2007. Susceptibility to erythromycin, clindamycin, and tetracycline was determined for all invasive isolates during 2004-2007 and to levofloxacin for isolates during 2005-2007. In addition to this scheme, other typing methods such as PFGE and superantigen profiling were used selectively for determining the clonal relationship of isolates.

In the case-control study, isolates of group A (*S.pyogenes*) and group G (*S. dysgalactiae* subsp. *equisimilis*) streptococci were characterised by T serotyping, *emm* typing, and PFGE.

Table 5. Characterisation of bacterial isolates in the publications of this work by different sero- and genotyping methods.

Publication	Years	Isolates characterised by method				
		T serotyping	***emm* typing**	**Antimicrobial susceptibility (ery, cli, tet, levo)**	**PFGE**	**SAg profiling**
I	1995-2004	all	all T28 isolates	ND[a]	ND	ND
II	2005-2007	2005-6: all 2007: selected[b]	all	all	all *emm*84 isolates	selected *emm*84 isolates[c]
III	2004-2007	-[d]	all	all[e]	ND	ND
IV	2004-2005	all	all	ND	all	ND

[a] ND, not determined

[b] 5 selected *emm*84 isolates from 2007

[c] 10 selected *emm*84 isolates from 2005-2007

[d] results not included in the publication

[e] susceptibility to levofloxacin not determined in 2004.

4.3.1 T serotyping

T serotyping was performed using the available anti-T agglutination sera: five polyvalent and 21 monovalent sera (1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 18, 22, 23, 25, 27, 28, 44, B3264, and Imp19) (Sevac) according to standard procedure [224].

4.3.2 *emm* typing

emm typing was performed according to the Centers of Disease Control and Prevention guidelines [45]. The *emm* gene was amplified using either of two sets of primers: MF1 and MR1, and primer1 and primer2 (Table 6). Until 2005, the primer set used was MF1 and MR1. Primer1 and primer2 were introduced in 2006 to improve the method and minimise the amount of non-typable isolates. MF1 and MR1 remained as a secondary choice if the *emm* gene could not be amplified with the primer1 and primer2.

With primers MF1 and MR1, the following PCR conditions were used: initial denaturation at 95°C for 10 min and 94°C for 3 min, 35 cycles of denaturation at 93°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 2 min, with a final extension step at 72°C for 10 min. With primer 1 and primer 2, PCR conditions were as follows: initial denaturation at 95°C for 10 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 46°C for 1 min, and extension at 72°C for 2.5 min; and 1 final extension cycle at 72°C for 7 min [310]. PCR products were purified with QIAquick PCR Purification Kit (Qiagen) as described by the manufacturer.

Table 6. Primers used for emm typing.

Primer	Sequence 5' -> 3'	Reference
MF1 (forward and sequencing)	ATA AGG AGC ATA AAA ATG GCT	[153]
MR1 (reverse)	AGC TTA GTT TTC TTC TTT GCG	[153]
primer 1 (forward)	TAT T(C/G)G CTT AGA AAA TTA A	[17, 47]
primer 2 (reverse)	GCA AGT TCT TCA GCT TGT TT	[17, 47]
emmseq2 (sequencing)	TAT TCG CTT AGA AAA TTA AAA ACA GG	[47]

The *emm* sequencing reaction was performed with primer MF1 or emmseq2 with use of BigDye chemistry (Applied Biosystems), with cycling times of 30 cycles of denaturation at 96°C for 20 s, annealing at 55°C for 20 s, and extension at 60°C for 4 min. Sequence data were analysed using ABI Prism 310 Genetic Analyzer (Applied Biosystems) and the sequences obtained compared against the CDC *Streptococcus pyogenes emm* sequence database to assign *emm* types [45].

4.3.3 Pulsed field gel electrophoresis (PFGE) analysis

PFGE was performed for selected isolates in studies II and IV using standard methods [290]. Bacterial cells were lysed with lysostafin, DNA was digested with SmaI (Roche), and restriction fragments were separated with CHEF-DR II (Bio-Rad) for 23 h, with pulse times of 10-35 s. Bionumerics software (Applied Maths) was used for analysing restriction profiles, which were interpreted along general guidelines [313]. Strains with ≥90% similarity were considered as the same PFGE type; strains with ≥80% similarity were considered as related types. Types were designated by uppercase letters (for GAS) or Roman numerals (for GGS). Individual strains were termed "unique".

4.3.4 Superantigen profiling

Superantigen profiling was performed for selected isolates in study II. Two multiplex PCR reactions were performed for detection of pyrogenic exotoxin genes *speA*, *speB*, *speC*, *speF*, *speG*, *speH,* and *speJ*, and the streptococcal superantigen *ssa* gene. A single PCR was used to detect streptococcal mitogenic exotoxin *smeZ* gene [72, 268].

4.3.5 Antimicrobial susceptibility testing

Susceptibility to erythromycin, clindamycin, tetracycline, and levofloxacin was determined for invasive strains in studies II and III by agar dilution. Susceptibility was assigned as the minimum inhibitory concentration (MIC) of a given antimicrobial according to interpretative criteria for *Streptococcus* spp. (other than *S. pneumoniae*) as recommended by the Clinical and Laboratory Standards Institute (CLSI) [54]. The MIC was assigned as the lowest antimicrobial concentration completely inhibiting bacterial growth. The breakpoints used for the susceptible (S), intermediate (I), and resistant (R) categories for these antimicrobials were as follows: erythromycin: S ≤ 0.25 mg/l, I = 0.5 mg/l, R ≥ 1 mg/l; clindamycin: S ≤ 0.25 mg/l, I = 0.5 mg/l, R ≥ 1 mg/l; tetracycline: S ≤ 1 mg/l, I = 2 mg/l, R ≥ 4 mg/l; and levofloxacin: S ≤ 1 mg/l, I = 2 mg/l, R ≥ 4 mg/l.

4.4 Data analysis and statistics

Data on the cases of invasive GAS infection, their outcome, and isolate characterisation data (where available) were linked using the national identity code of the patient and the date of specimen. Where the national identity code was not available (prior to 2004), other available information was used for linkage, such as the date of specimen, date of birth of patient and place of treatment. Population data were obtained from Statistics Finland. Average annual incidence rates were

calculated for 1998-2007 using the population census at the end of the previous year as the denominator. Age- and sex-specific incidence rates and male-to-female ratios with 95% confidence intervals (CI) were calculated. Case fatality rates (CFR) were calculated for 2004-2007 as the number of fatal cases compared to the total number of cases for each year. Case fatality by *emm* type was calculated in a similar manner by dividing the number of fatal cases per type with the total number of isolates for each *emm* type separately. For the cellulitis study, only one episode per patient was considered when calculating percentages, unless otherwise specified. In clinical samples (skin, blood), a patient was considered culture positive for a given bacterial group if the culture sample was positive for that bacterial group at any time during the study.

Incidence rates and CIs according to the Poisson distribution were calculated using R statistical software version 2.9.0 (R Development Core Team). Data were analysed using Intercooled Stata™ 9.1 software for Windows (StataCorp) and GraphPad software [120]. Fisher's exact, χ^2, and Kruskal-Wallis tests were applied where appropriate to test for statistical significance between subgroups and distributions. McNemar's test was used in the case-control study for the comparison of differences between pharyngeal bacterial findings of patients and control subjects. Differences were considered significant when $P<0.05$.

4.5 Ethical considerations

Finnish clinical laboratories mandatorily notify *S. pyogenes* isolations from blood or CSF to the NIDR. THL conducts infectious disease surveillance and research by legislation and has the rights to use data from the NIDR and the outcome data from the Population Information System for that purpose. The previously unpublished incidence data presented in this book were retrieved from the public user interface of NIDR [316].

The patients recruited for the acute cellulitis study signed a declaration confirming their informed consent. The acute cellulitis study was approved by the Ethical Review Board of Pirkanmaa District, Tampere University Hospital, Finland.

5 RESULTS

5.1 The epidemiology of invasive GAS disease in Finland (I, II, III)

The epidemiology of invasive GAS disease in Finland was studied in detail in the publication III, which covered the years 1998-2007. Average annual incidence rates from 1995-2004 and 2005-2007 were also presented in publications I and II, respectively. An overview of the received notifications and isolates is presented in Table 7.

Table 7. An overview of the number of notifications and isolates included in the published works. Data for 2008 retrieved from NIDR [316].

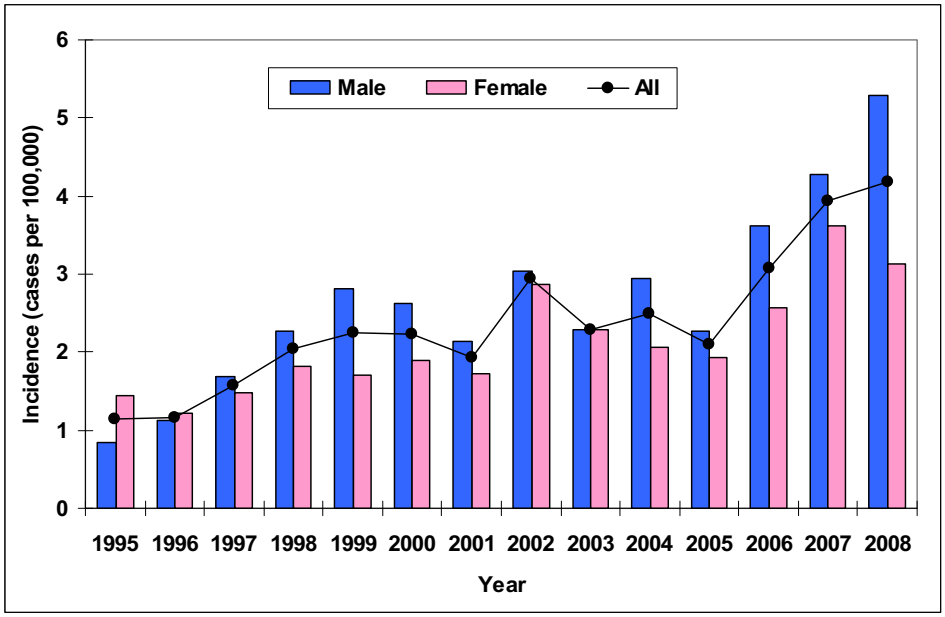
Year	No. of notifications received	No. of isolates received	Publication
1995	58	43	I
1996	60	58	I
1997	80	79	I
1998	105	110	I, III
1999	116	113	I, III
2000	116	103	I, III
2001	100	93	I, III
2002	153	138	I, III
2003	119	118	I, III
2004	130	128	I, III
2005	110	110	II, III
2006	162	161	II, III
2007	207	203	II, III
2008	221	NA[a]	NA
Total	1737	1457	NA

[a] NA, not applicable.

5.1.1 Annual incidence (I, II, III)

During 1995-2007, the annual incidence rate fluctuated in the range of 1.1-3.9 cases per 100,000 population. Despite fluctuation, an increasing trend was evident, as shown in Figure 2. Peaks occurred in 2002 (2.9 cases per 100,000) and in 2006-2007 (3.1-3.9). The year with the lowest incidence was 1995 (1.1) and troughs also occurred in 2001 (1.9) and 2005 (2.1). The incidence continued to increase in 2008 (4.2 cases per 100,000) [316].

Figure 2. Annual incidence rate of iGAS in Finland, 1995-2008. Data for 2008 retrieved from NIDR [316].



5.1.2 Incidence by district (III)

The incidence varied considerably between the 20 healthcare districts, and from year-to-year within the districts, owing to the small number of cases observed. Rates were therefore examined at the level of tertiary care districts, which include 2-5 healthcare districts within each of the five tertiary districts. Between these tertiary care districts, the average annual incidence rate varied from 1.8-3.1 cases per 100,000 population during 1998-2007. The annual incidence rate increased in all districts by more than 1 case per 100,000 and more than doubled in three out of five of these districts. Fourty-one per cent of all cases occurred in the tertiary care district around the capital city Helsinki (with the largest population), where the annual incidence rate varied within the range of 2.2-4.4 cases per 100,000.

5.1.3 Age- and sex-specific incidence (III)

A total of 1318 cases of iGAS (range by year, 100-207) were identified during 1998-2007; the median age of case-patients was 52 years (range 0-95 years). Fifty-five percent of the case-patients (N= 719) were male. During the study, the average annual incidence rate of iGAS was 2.5 cases per 100,000 population.

The average incidence rate was higher in males (2.8) than in females (2.2) and it increased by age in adult age groups (Table 8). The highest rates were seen in the elderly (≥65 years of age), while the lowest rates were seen in the age groups of 1-24 years. The rate in males was significantly higher than females especially among patients aged 45-64 years. In contrast, females had a significantly higher incidence than males among patients aged 25-34 years.

Table 8. Incidence of invasive group A streptococcal disease by age and sex, Finland, 1998-2007.

Age group (years)	Rate[a] of invasive disease			Incidence rate ratio (RR)[b]	95% CI[c] of RR
	Male (N=719)	Female (N=599)	Total (N=1318)		
<1	2.7	2.2	2.5	1.3	0.4-3.9
1-14	1.2	0.9	1.0	1.3	0.8-2.0
15-24	0.8	1.2	1.0	0.6	0.4-1.0
25-34	1.2	2.7	1.9	0.4	0.3-0.6*
35-44	3.0	2.7	2.8	1.1	0.9-1.5
45-54	4.2	1.8	3.0	2.4	1.8-3.2*
55-64	4.8	2.4	3.6	2.0	1.5-2.6*
≥65	5.1	3.8	4.3	1.4	1.1-1.7*
All	2.8	2.2	2.5	1.3	1.1-1.4*

[a] Average incidence (cases per 100,000 population)

[b] male-to-female ratio

[c] CI, confidence interval. Statistical significance (P<0.05) is indicated with an asterisk (*).

Males had a higher incidence than females in all study years except 2003, when the rates were equal (Table 9). The incidence rate ratio (male-to-female) showed a statistically significant difference in 1999 (RR, 1.7) and bordered on significant in 2004 (1.4) and 2006 (1.4).

Table 9. Annual incidence of invasive group A streptococcal disease, Finland, 1998-2007.

Year	Rate[a] of invasive disease (no. of cases)						Incidence rate ratio (RR)[b]	95% CI[c] of RR
	Male		Female		Total			
1998	2.3	(57)	1.8	(48)	2.1	(105)	1.2	0.9-1.8
1999	2.8	(71)	1.7	(45)	2.2	(116)	1.7	1.1-2.4*
2000	2.6	(66)	1.9	(50)	2.2	(116)	1.4	1.0-2.0
2001	2.1	(54)	1.7	(46)	1.9	(100)	1.2	0.8-1.8
2002	3.0	(77)	2.9	(76)	2.9	(153)	1.1	0.8-1.5
2003	2.3	(58)	2.3	(61)	2.3	(119)	1.0	0.7-1.4
2004	2.9	(75)	2.1	(55)	2.5	(130)	1.4	1.0-2.0
2005	2.3	(58)	1.9	(52)	2.1	(110)	1.2	0.8-1.7
2006	3.6	(93)	2.6	(69)	3.1	(162)	1.4	1.0-1.9
2007	4.2	(110)	3.6	(97)	3.9	(207)	1.2	0.9-1.6
All	2.8	(719)	2.2	(599)	2.5	(1318)	1.3	1.1-1.4*

[a] Average annual incidence (cases per 100,000 population)

[b] male-to-female ratio

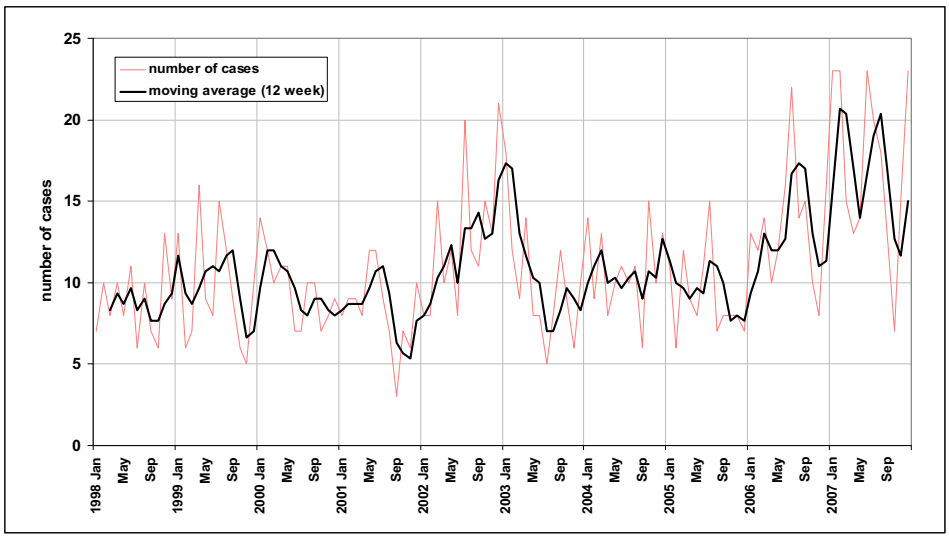
[c] CI, confidence interval. Statistical significance ($P<0.05$) is indicated with an asterisk (*).

5.2 Seasonal patterns of infection (III)

Seasonal patterns of iGAS infection were examined during 1998-2007. The monthly number of cases varied in the range of 3-23, depending on the overall incidence. In general, the months with most cases were January, July and December, whereas the months with the least cases were September, October and November. Figure 3 shows the number of iGAS cases over time. A twelve-week moving average was used to smooth variation by chance due to relatively small number of cases per month and in order to obtain a clearer trendline.

Two peak seasons of iGAS disease could be identified, during the midsummer (June-August) and the midwinter (December to February). The peaks during the summer were more evident than those during the winter. Both males and females had peaks of cases during the winter, whereas the summer peak occurred predominantly for males. The overall trend for both sexes was that the least amount of iGAS cases occurred during the autumn months (September-November).

Figure 3. The monthly number of cases of invasive group A streptococcal disease in Finland during 1998-2007.Red line, number of cases; black line, number of cases (12-week moving average).



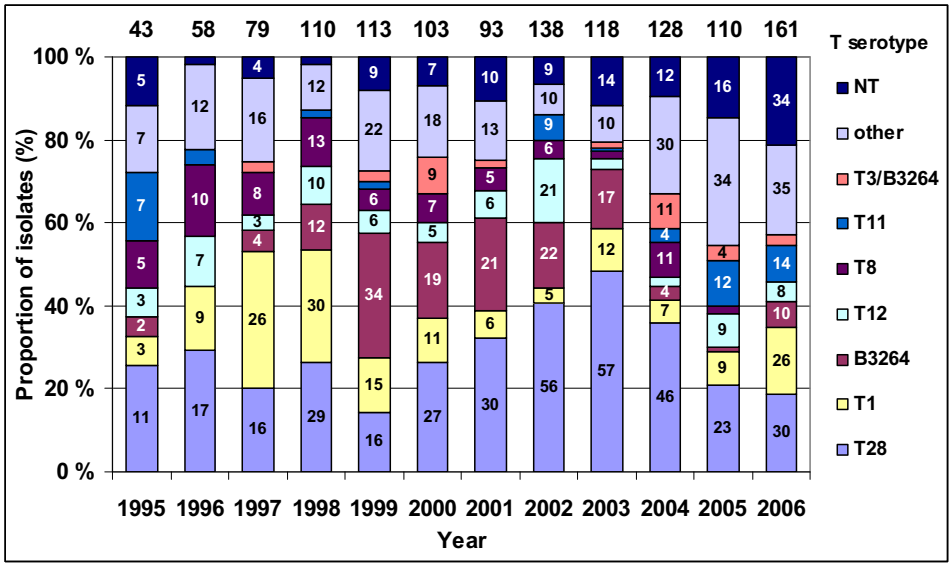
5.3 T- and *emm* type prevalence (I, II, III)

The typing data from 1995-2003 presented in this book and in publication I is based on referred isolates. This number differs slightly from the amount of notifications for the same time period. For a small proportion of notifications a matching isolate was missing; also, for some referred isolates a matching notification did not exist. For the data during 2004-2007, and for publications II and III, only isolates that were matched to notifications were included in the analyses. Therefore, the number of isolates in 2004 presented in this book differs slightly to the number presented for 2004 in publication I, which was based on non-matched data.

During 1995-2003, 855 isolates and during 2004-2007, 602 isolates were received for characterisation, amounting to 1457 iGAS isolates in total. T typing results of 1995-2004 were published in the original publication I; results from 2005-2006 are previously unpublished. The most common T serotypes encountered in Finland during 1995-2006 in order of prevalence were T28 (29%), T1 (13%), TB3264 (12%), T12 (7%), T8 (6%), T11 (4%), and T3/B6326 (3%) (Figure 4). Depending on the year, the seven most common types accounted for 55-87% of the isolates. The annual proportion of nontypable (NT) isolates varied within a range of 2-21%.

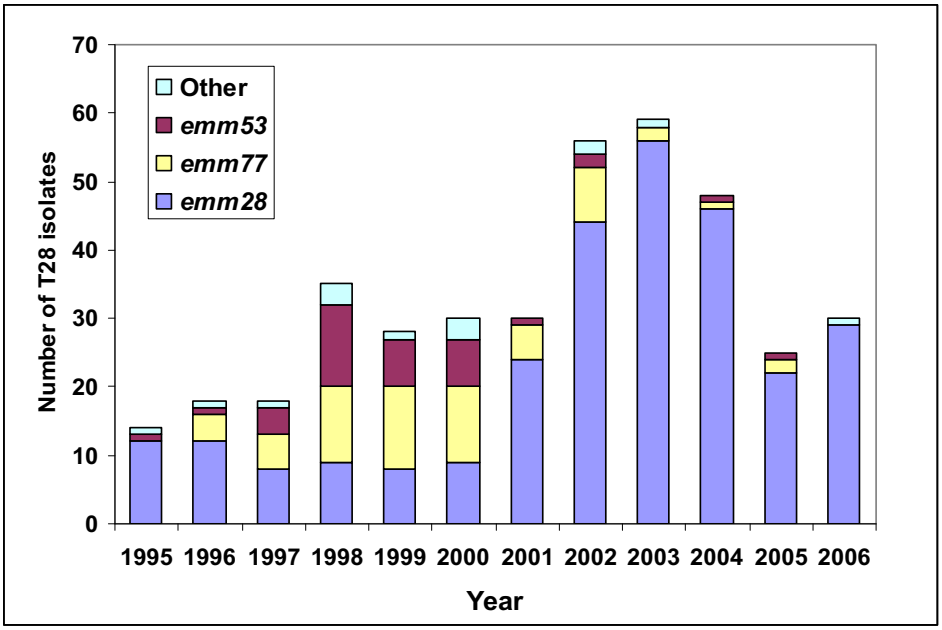
During the study period, the prevalence of common T types was constantly changing. First the T1 strains peaked in 1997-1998, after that the prevalence of TB3264 strains was the highest in 1999, and the T28 strains had a peak later in 2003, this type comprising almost half of the iGAS isolates at that time. The prevalence of other types seemed to go through less dramatic changes. It is also noteworthy that the proportion of nontypable strains increased to over 20% by 2006.

Figure 4. The proportions of most common T serotypes of Finnish iGAS isolates during 1995-2006. The number of isolates received and typed each year is indicated with numbers above the bars. The numbers in the bars represent the number of isolates for each serotype (indicated for the most common types). Years 1995-2003, referred isolates; 2004-2006 referred isolates matching to notifications. Data from 2005-2006 are previously unpublished.



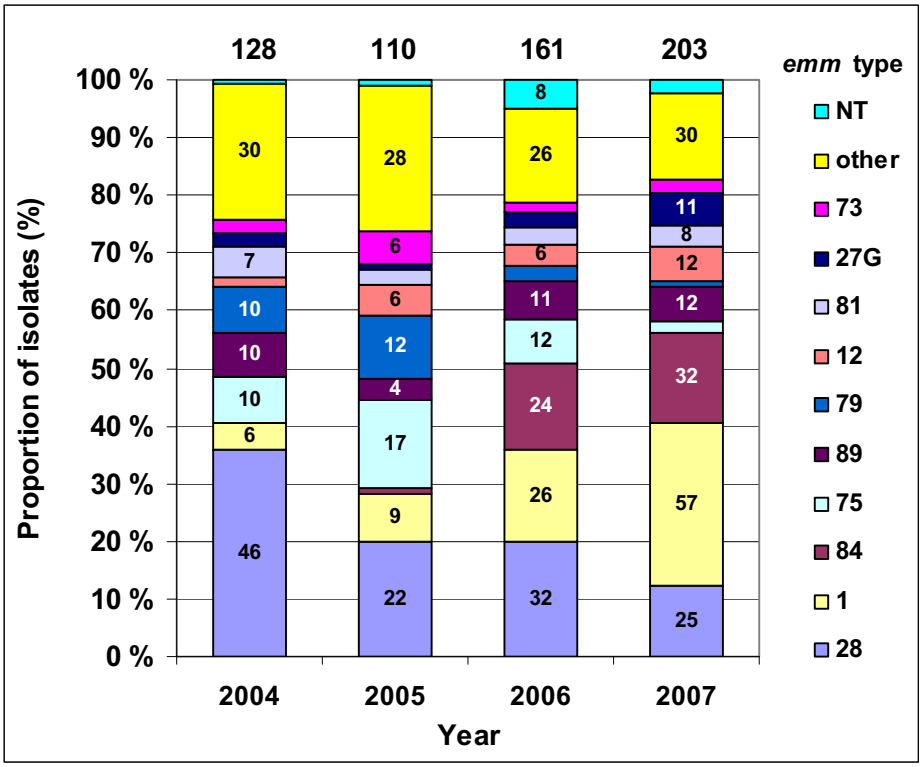
Serotype T28 strains, being the most common type overall during this study period, were studied in more detail in publication I. The *emm* typing results of all isolates of serotype T28 during 1995-2006 showed that among isolates that reacted with T28 antiserum alone or with T28 in combination with other T typing sera (N=391), six different *emm* types were found: *emm*28 (71.5% of all T28), *emm*77 (15.5%), *emm*53 (9.5%), *emm*2 (2%), *emm*87 (1%), and *emm*4 (0.5%). Figure 5 illustrates how the amount of these *emm* types among T28 strains fluctuated over time. The isolates reacting with T28 antiserum were more heterogeneous during 1997-2000, and less heterogeneous in all other years when the isolates were mostly of genotype *emm*28.

Figure 5. The emm types of serotype T28 isolates during 1995-2006 in Finland. Isolates include strains reacting with T28 antiserum alone (N=358) and T28 in combination with any other T typing sera (N=33). Other types include emm2, emm87 and emm4. Data from 2005-2006 are previously unpublished.



Prior to 2004, the iGAS isolates were selectively characterised by *emm* typing, but comprehensive *emm* typing data for all isolates exists for 2004-2007. A total of 46 *emm* types among 602 isolates were encountered during this time. The proportion of nontypable isolates varied annually from 1-5%. The most common *emm* types during 2004-2007 in the order of prevalence were 28 (21%), 1 (16%), 84 (10%), 75 (7%), and 89 (6%), which together accounted for 48-65% of the isolates depending on the year (Figure 6). In 2004, type *emm*28 was the most predominant type, and its proportion gradually declined each year while that of *emm*1 increased, and by 2007 *emm*1 had become the most common type. In 2005, an uncommon type, *emm*84, emerged in Finland and became increasingly prevalent, ranking as the second most common type by 2007. Again, similarly to T serotypes, the less common *emm* types showed less fluctuation than the most common types.

Figure 6. Ten most common emm types of Finnish iGAS isolates during 2004-2007. The number of isolates received and typed each year is indicated with numbers above the bars. The numbers in the bars represent the number of isolates for each emm type (indicated for the most common types).



A comparison of the *emm* and T typing patterns could be undertaken during the period 2004-2006, when these methods were used in unison. Table 10 contains all *emm* and T type combinations encountered in Finland during these three years, and shows relationships between *emm* and T types as well as differences in Finnish type combinations in comparison to those obtained by others [159, 200].

Table 10. The emm and T types of invasive GAS isolates collected in Finland during 2004-2006. T patterns that have been commonly observed by others [159, 200] are indicated by bold, underlined font; patterns closely related to these commonly found T patterns are indicated by underlined font. Closely related T patterns are separated by commas, distinctly different T patterns are separated by semicolons. Table design adapted from [159].

***emm* type**	**No. of isolates**	**No. of T/*emm* combinations**	**T type (no. of isolates)[a]**
1	41	1	**1** (41)
3	1	1	3
4	8	2	**4** (5); NT[b] (3)
11	2	2	**11** (1), 8/11 (1)
12	14	1	**12** (14)
22	3	2	**12** (1); NT (2)
25	1	1	**1**
27G	8	2	5/27 (6); NT (2)
28	100	2	**28** (97); NT (3)
44/61	1	1	8/11
53	2	2	3/B3264 (1); 28 (1)
59	1	1	NT
60	2	1	**4** (2)
66	3	2	**12** (2), 3/12 (1)
67	1	1	3
68	1	1	3/B3264
69	1	1	3
73	12	6	3/13 (4), 13 (3), 13/B3264 (1), **3/13/B3264** (1), B3264 (1); NT (2)
75	39	4	8/25 (19), 25 (17), **8/25/Imp19** (2), 25/Imp19 (1)
76	1	1	**12**
77	9	6	**13/28** (3); Imp19 (2), 8/Imp19 (1), 25/Imp19 (1); **13** (1); NT (1)
78	3	3	**11** (1); 3/B3264 (1); NT (1)
79	26	5	**11** (11); 8 (5); 12 (1); B3264 (1); NT (8)
81	15	4	8 (4); B3264 (3), 3/B3264 (2); NT (6)
82	1	1	5/27
83	1	1	8
84	25	4	11 (9); 3 (2), 3/B3264 (1); **NT** (13)
85	9	5	**3** (4), 9/3 (2), 3/13 (1), 9/3/13 (1); NT (1)
87	1	1	**28**
89	25	4	3/B3264 (14), B3264 (6); 8 (1); NT (4)

102	1	1	3
104	1	1	8/11
108	1	1	**NT**
110	13	5	11 (5), 5/11 (1); 8 (1); B3264 (1); **NT** (5)
112	5	3	**8** (1); 11 (3); NT (1)
118	1	1	13
122	1	1	NT
124	2	1	NT
st75	2	1	25
st369	1	1	14
st1389	1	1	NT
st3850	1	1	NT
st554	1	1	NT
stM3	1	1	3
NA[c]	10	6	B3264 (3), 3 (2), 3/13 (1), 3/B3264 (1), 3/13/B3264 (1); NT (2)
Total	399	95	-

[a] The number of isolates of each specific T/*emm*-type combination is described within brackets.

[b] NT, nontypable.

[c] NA, *emm* type not available (nontypable).

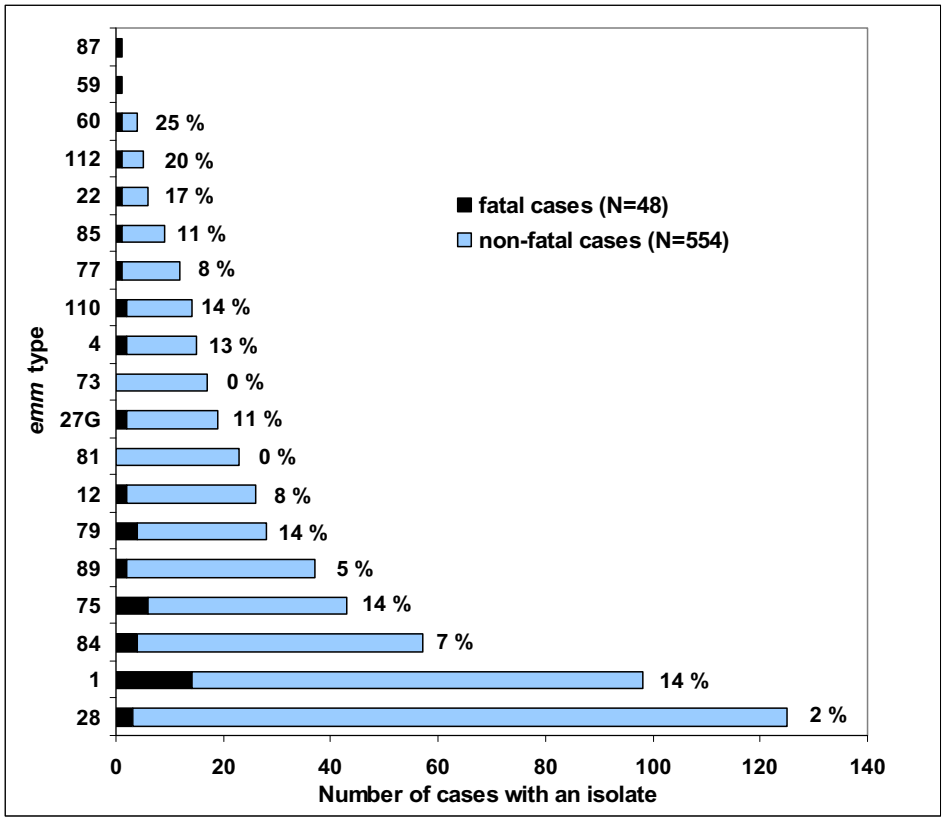
Because of the relatively small numbers of cases, analyses of associations of specific *emm* types and age or sex of the patient were limited to the most common *emm* types. Type *emm*28 infections were significantly overrepresented in females compared to males (55% vs. 45%, respectively; P<0.01). The same was true for type *emm*4 (73% vs. 27%, respectively, P<0.05). More specifically, a significantly larger proportion of infections by *emm*28 were concentrated in females of child-bearing age (15-44 years) than males of the same age (48% vs. 21% of *emm*28 infections, respectively; P<0.01).

5.4 Outcome of invasive infections (II, III)

During 2004-2007, 48 of a total of 609 case-patients died within 7 days of positive blood or CSF culture, corresponding to an overall case fatality of 8%. There was no significant difference in the case fatality rate in males and females (9% vs. 6%, P=0.172). The highest case fatality rate occurred in 2005 (12%), followed by 2006 (10%), while the year with the lowest rate was 2004 (3%). Case-patients with a fatal outcome were older than those who survived the infection (median age, 60 vs. 54 years; P<0.01). There were no fatalities in the age groups of <1 year and 15-24 years.

The outcome by *emm* type is presented in Figure 7. Infections by type *emm*1 were significantly associated with a high case fatality rate (14% vs. 8% in all other types; P<0.02). Infections caused by *emm*75 and 79 were also associated with a higher than average case fatality (14% for both types). In contrast, the case fatality associated with type *emm*28 infections was low (2% vs. all other types, P<0.01), also true for type 81 (0%, respectively). The *emm* types that contributed most to the case fatality were *emm*1 (29% of all fatal cases; range by year 15-47%), followed by *emm*75 (13%, range 0-25%), *emm*79 (8%, range 6-25%), and *emm*84 (8%; range 0-20%). Case fatality in *emm*84 infections did not significantly differ to that of all other types (7% vs. 8%, respectively).

Figure 7. Distribution of emm types causing iGAS infections according to 7-day case fatality, 2004-2007, Finland. Thirteen most common emm types regardless of outcome, and all rarer types(N=6) causing at least one fatal case are included. The percentages indicate case fatality by emm type (shown for types causing two or more infections).



5.5 Superantigen profiles in relation to PFGE strain types (II)

In order to obtain more information about the clonality and virulence factors of *emm*84 isolates, their PFGE and superantigen profiles were studied. PFGE profiling was performed for all *emm*84 isolates during 2005-2007 (N=57). These isolates fell into six PFGE strain types, A1 (52 isolates) and A2, B1, B2, C, and D (1 isolate each), with related strain types of A1 and A2, and B1 and B2, respectively. The strain type A1 predominated until 2007, when sporadic cases with other strain types emerged among the *emm*84 strains. Ten *emm*84 isolates representing different PFGE strain types were selected for superantigen profiling (Table 11). Variability in the T types and SAg gene content existed between *emm*84 isolates of different PFGE strain types, but the results of these methods were mainly in concordance, as related strain types harboured a similar set of SAg genes.

Table 11. Characteristics of invasive GAS isolates of type emm84, as determined by PFGE, T serotyping and SAg profiling.

PFGE strain type	No. of isolates (fatal)	T serotype(s)	SAg profile								
			speA	***speB***	***speC***	***speF***	***speG***	***speH***	***speJ***	***ssa***	***smeZ***
A1	52 (3)	NT[a]; T11; T3; T3/B3264[b]	-	+	-	+	+	-	+[c]	-	-
A2	1 (0)	T11/B3264	-	+	-	+	+	-	-	-	-
C	1 (0)	T11/B3264	-	+	-	+	+	-	-	-	-
D	1 (0)	T8/25/Imp19	-	+	-	+	+	-	-	-	+
B1	1 (0)	T1	+	+	-	+	+	-	+	-	+
B2	1 (1)	T1	+	+	-	+	+	-	+	-	+

[a] NT, nontypable.

[b] Results from isolates from 2005 to 2006 (N=25), presented in the order of prevalence.

[c] *speJ* was present in 3/5 isolates selected for analysis.

5.6 Antimicrobial susceptibility (II, III)

All iGAS isolates during 2004-2007 (N=602) were tested for antimicrobial resistance to erythromycin, clindamycin, and tetracycline; isolates of 2005-2007 were also tested for levofloxacin. Overall, 1.5% of the isolates were resistant to erythromycin, 0.5% to clindamycin, and 16% to tetracycline; tetracycline resistance varied depending on the year (Table 12). The tetracycline resistant strains were mainly *emm* types 81 (20 resistant isolates in total; 87% of this type resistant to tetracycline), 27G (19; 100%), 77 (12; 100%), and 85 (9; 100%). In contrast, resistance to tetracycline was not common among the most prevalent *emm* types, such as 28 (4% of isolates of this type resistant to tetracycline), 1 (0%), 84 (2%), 75 (2%), 89 (0%), 79 (0%), and 12 (0%).

Table 12. Resistance of iGAS isolates to antimicrobials, 2004-2007.

Antimicrobial agent	Resistance (%)			
	2004 N=128	2005 N=110	2006 N=161	2007 N=203
Erythromycin	1.6	1.8	0.6	2.0
Clindamycin	0.8	0.0	1.2	0.0
Tetracycline	22.7	15.5	12.4	15.3
Levofloxacin	ND[a]	0.0	0.0	0.0

[a] ND, not determined.

5.7 Investigation of a cluster of infections (II)

Infections of type *emm*84 were primarily located in the largest healthcare district of the Helsinki metropolitan area, where type *emm*84 was the most common type in 2006 and 2007. In 2006, these infections were treated in several hospitals, one of which had a cluster of cases occurring during April-October involving seven patients. The isolates were of the major PFGE strain type A1 (Table 11).

Investigation of medical records of these patients revealed that they had no common exposure or contacts. All patients had one or more predisposing factor for invasive infection, such as traumatic wound, diabetes, intravenous drug use, alcoholism, immunodeficiency, and malignancy. None of these infections had fatal outcome.

5.8 Acute bacterial, nonnecrotizing cellulitis in Finland (IV)

A total of 90 patients presenting with 98 episodes of acute bacterial cellulitis were included in the study during April 2004-March 2005. Males dominated among the patients (58 men vs. 32 women); the median age of the patients was 58 years (range,

21-90 years). Correspondingly to the patients, 90 control subjects and 38 family members were recruited in the study.

More cellulitis episodes occurred during the months of July-September than during any other three-month period ($P<0.05$). Sixteen of the 98 episodes could be classified as classic erysipelas, the form of cellulitis presenting with a clearly demarcated area of inflammation (Figure 8).

Figure 8. A classic erysipelas infection in the leg with a clearly defined area of inflammation. The photograph was kindly provided by a participant in the cellulitis study.

Antimicrobial treatment had been initiated in 28% of disease episodes prior to sampling. A skin swab sample was taken from 66 patients presenting with a total of 73 disease episodes. No skin swab was taken from the rest of the patients (N=24), as the skin in the infection focus was intact and no other abrasion or wound could be found. Excluding recurrent episodes, a total of 24 patients harboured β-haemolytic streptococci: GGS (17 patients), GAS (5), GGS+GAS (1), and GBS (1). Additionally, two patients (2%) were blood culture positive for GGS. *S. aureus* was isolated from 27 patients, either alone (N=10) or concomitantly with β-haemolytic streptococci (N=17). All of the GGS were of the species *S. dysgalactiae* subsp. *equisimilis*.

Of the 24 culture positive samples for β-haemolytic streptococci, 9 were isolated from the infection focus and 15 from the suspected site of entry (wound, intertrigo etc.). Among the skin swab and blood culture samples, eleven *emm* types among 20 GGS isolates and four *emm* types among six GAS isolates were found (Table 13). PFGE analysis of the isolates revealed that none of the strains isolated from different patients were identical, with the exception of the *emm*81 isolates, which were part of a cluster of cases in a nursing home. Thus, no clear predominance of a specific *emm* type was seen.

Table 13. emm types of group G and A streptococci isolated from acute bacterial cellulitis. Excluding recurrences; only one isolate of a streptococcal group per patient is included.

Group antigen	*emm* type	No. of isolates	Sample site
G	stC74A	1	skin
G	stC6979	2	skin; blood
G	stG6.0	2	skin
G	stG6.1	2	skin
G	stG11	1	skin
G	stG166b	2	skin
G	stG245	2	skin
G	stG480	2	skin
G	stG485	2	skin; blood
G	stG643	3	skin
G	stG5420	1	skin
A	*emm*28	1	skin
A	*emm*73	1	skin
A	*emm*81	3	skin
A	*emm*85	1	skin

5.7.1 Recurrent infections (IV)

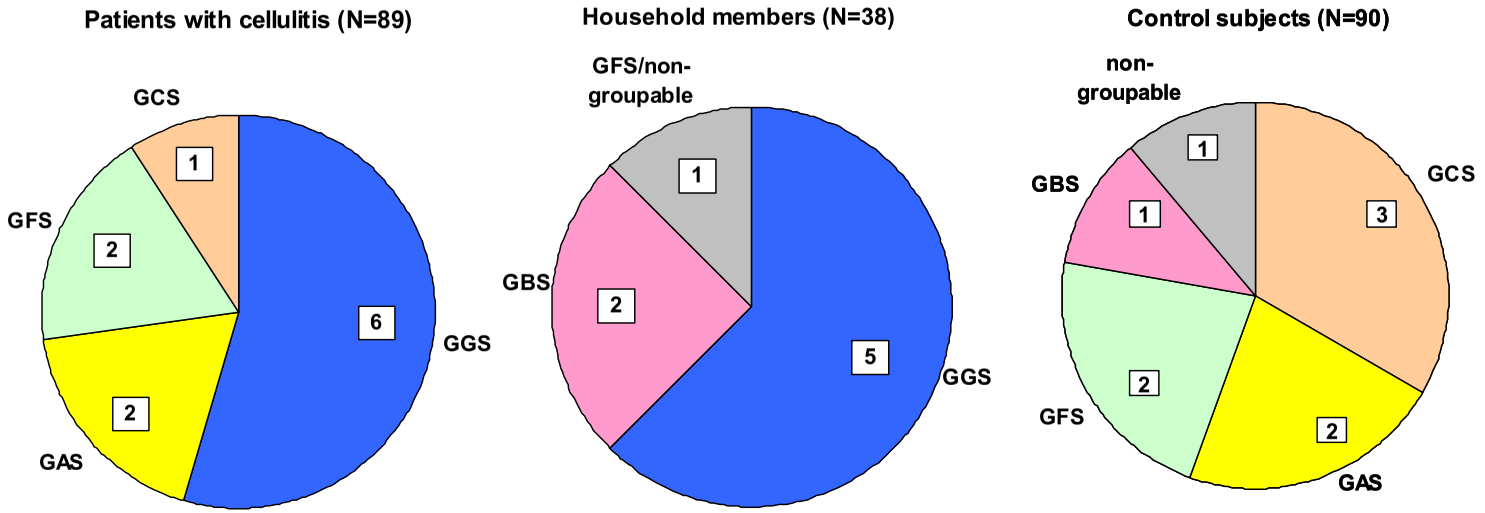
Six patients (five males, one female) had recurrent episodes during the study; four patients had two episodes and two patients had three episodes. The median age of these patients was 48 years. The time between recurrences varied between 46 and 156 days (median 81 days). All of these patients had a history of at least one previous cellulitis infection. GGS was isolated from three of these patients, concomitantly with *S. aureus*. In two patients, the same strain of GGS (*emm* types stG6.0 and stG11) with identical PFGE profile was isolated from two consecutive episodes.

5.7.2 Pharyngeal carriage (IV)

Pharyngeal carriage of β-haemolytic streptococci was studied from the patients, their household members and control subjects. Throat swabs were obtained from all but one patient (N=89) and from all other study subjects (90 control subjects, 38 household members). β-haemolytic streptococci were present in the pharynx of 13% of patients, 21% of household members, and 10% of control subjects (Figure 9). Among these isolates, GGS was significantly more commonly found from patients than control subjects (7% vs. 0%, respectively; $P<0.04$). Thirteen percent of household members of the patients also harboured GGS. *S. aureus* was present in ≤10% of the samples of these groups.

One patient carried the same GGS strain of *emm* type stG245 with identical PFGE type III in the pharynx and affected skin. On two occasions, the same strain was shared within a household. Those were the following: one patient and a household member harboured identical clones of GGS type stG480 with PFGE type II in their pharynx, and two members of the same household shared the same clone of GGS stG6.1.

Figure 9. The number of pharyngeal samples culture positive for β-haemolytic streptococci in the three study groups. N, the total number of samples taken in each study group. In patients with recurrent episodes only the first episode with the corresponding sample was included. The total number of isolates for each bacterial group is shown within boxes. GAS, GBS, GCS, GFS, and GGS, group A, B, C, F, and G streptococcus, respectively.



6 DISCUSSION

This study describes the epidemiology of invasive *S. pyogenes* infections in Finland over more than a decade-long period of nationwide population-based surveillance. Overall, annual and age- and sex-specific incidence rates were calculated. Seasonal patterns and outcome due to these infections were studied. The study size of over 1500 cases of iGAS disease accumulated over the years provided a large enough sample for scientific analysis. Bacterial cellulitis infections caused by β-haemolytic streptococci were studied in the first population-based clinical study within a defined region in Finland. The *S. pyogenes* isolates causing invasive disease and cellulitis were characterised with both conventional and molecular typing methods. More detailed analyses were undertaken on isolates representing the most common type (T28) and one emerging type (*emm*84). The main findings and limitations of this study are discussed below.

6.1 The epidemiology of invasive *S. pyogenes* disease in Finland

This study has shown that the incidence of iGAS disease in Finland fluctuated since the 1990s but had an increasing trend. The increase was especially steep during the later years, from 2006 onwards. The incidence rate of 3.9 cases per 100,000 population observed in 2007 was the highest since 1995, with further increases noted beyond this study in 2008, reaching 4.2/100,000 [316]. During 2003-2004, the UK, Sweden, Denmark, and Finland had the highest rates of infection (range 2.5-3.3 per 100,000) in Europe [185]. Indications of a very recent increase in incidence can be seen in the UK and Sweden, whereas the rate has remained relatively stable in the USA during this decade [46, 183, 244, 287]. It remains to be seen if the present influx will be a temporary phenomenon or if the number of these infections will continue to increase in Finland. Although invasive *S. pyogenes* infections are still quite rare, the trend is worrying, especially as means of preventing these infections are limited.

Comparisons of incidence rates are complicated by differences in case definitions between countries. The surveillance scheme of invasive GAS disease in Finland includes, according to the case definition, only cases with a blood or CSF isolate. Non-bacteraemic cases with other sterile site isolations, including those who develop life-threatening complications such as STSS are excluded. This may partly explain the lower incidence rate observed in Finland, as in many other countries a broader definition of iGAS disease is used. Furthermore, differences between the healthcare districts in terms of blood culture sampling practices, diagnostic

procedures, and use of automated blood culture instruments may also have had an effect on the incidence rate over time [176, 284].

Although it is known that individuals of all ages are affected by iGAS disease, the elderly and small children generally have the highest incidence. Also in Finland, the incidence generally increased with age, but the rate in the elderly patients (≥65 years of age) remained at a lower level than has been reported in Sweden, Denmark, the UK, and the USA [72, 184, 198, 244]. Similarly, the incidence in infants (<1 years) was similar to other Nordic countries and lower than in the UK, the USA, Canada, and Australia [72, 184, 198, 243, 244, 322].

Males had a higher incidence than females during this study. A slight male dominance in iGAS infections has also been noted by others [185, 188, 243, 244]. Differences in the age- and sex-specific rates could also be identified, with male patients being overrepresented among the patients aged 45-64 years. In contrast, more infections occurred in females than males aged 25-34 years. Earlier studies from Denmark and the UK noticed a similar peak in the incidence in females around the same age [184, 198]. Presumably there are many different factors contributing to these distinctions, such as sex-specific differences in risk factors, notably pregnancy, social behaviour (including the use of alcohol and drugs), occupation, and the threshold of seeking medical care in case of trauma.

Spatial and temporal variations in incidence, interspersed with occasional peaks, are known to characterise this disease and were also observed in this study [185, 244]. The incidence in the 20 healthcare districts varied considerably over time and in comparison to each other, partly due to the small population of some of these districts. The fluctuation in incidence is thought to be a reflection of dynamic changes in the *emm* type distribution, but also of population susceptibility to particular strains [72, 168, 242, 244]. The emergence of new types not previously encountered by a population may have a profound influence on the incidence.

The good coverage and efficient functioning of the national mandatory infectious disease notification system of Finland is a strength of this study. A corresponding isolate was received for approximately 94% of the notifications during 1995-2003 and for 99% of the notifications during 2004-2007. However, there are also some limitations to these findings. Firstly, the lower level of incidence during the first three study years (1995-1997) may be an artefact due to the transition into a new notification system in 1995, with notifications being potentially sub-optimal at first, affecting the coverage of the data. Thus, the actual incidence during these years may have been higher. However, from 1998 the notification procedure seems to have become well established and therefore the recent increase in incidence during the

2000s appears to be real and not due to improved notification. Secondly, the fact that the case-definition includes only bacteraemic cases influences the observed incidence, as some invasive infections (some GAS sterile site isolations and STSS) would be excluded. Finally, the surveillance system does not include collection of clinical data on disease manifestations, possible risk factors and underlying conditions. These data would be needed to address reasons behind the observed differences in the age- and sex-specific incidence. Recently, such a study has been undertaken within a defined area of one healthcare district in Finland, and earlier in 1995 through enhanced surveillance (unpublished) [256].

6.2 Molecular characteristics of invasive *S. pyogenes* strains

During this study, shifts in the type distribution of iGAS in Finland were seen; first with changes in T type and later *emm* type prevalence. Comparisons of results of T and *emm* typing were limited to the years 2004-2006 during which period both methods were used. Based on these results and observations by others, the T and *emm* typing results correlate especially well with type 1 (e.g. T1 strains are mostly M/*emm*1) and reasonably well with type 28 [99, 159]. In contrast, the isolates of types TB3264, T8, and T12 most likely represent a large pool of different *emm* types, similarly as the *emm* types 84, 75, and 89 are likely to represent several different T types. For this reason, and for ease of comparison to other countries, it was more rational to concentrate more on the recent *emm* typing instead of the T typing results.

Major changes occurred in the *emm* type distribution of iGAS in Finland during 2004-2007 when the most dominant type, *emm*28, was replaced by *emm*1. An overall increase in incidence of iGAS disease occurred in parallel to type *emm*1 increasing in prevalence, also observed in other studies [73, 96, 300, 305, 334]. However, changes in the prevalence of other *emm* types, such as the emergence of type *emm*84, may have also contributed to the increase in incidence.

The *emm* type distribution of iGAS isolates in Finland shares common features with that of other countries but also has unique characteristics. *emm* types 1, 28, and 89 are common globally but 75 and 84 are rarer and have not been mentioned among the five most common types elsewhere [72, 90, 150, 198, 243, 244, 322, 333]. Another interesting characteristic in the molecular epidemiology of Finnish GAS disease is low prevalence of infections by *emm*3, a common type in many other countries including Scandinavia, and often associated with high case fatality [150, 198, 219, 226, 242-244, 322, 333]. The reason for the near absence of *emm*3 is unclear, particularly as there has been some exposure to this type in Finland, judging by the small number of sporadic infections seen. The *emm* types that are included in

the putative 26-valent recombinant vaccine would have covered approximately 60% of the Finnish isolates in 2004, but slightly less than half of the isolates during the last two study years; this being a smaller coverage than has been estimated for the USA and Japan [150, 216, 244].

During the last two decades in Finland, a periodic fluctuation of some of the common types has occurred. Peaks in numbers of T/M/*emm*1 cases were observed in 1988-1990, 1997-1998, and 2006-2007 [139, 228]. In contrast, T/M/*emm*28 peaked in 1993-1995 and 2002-2004 [228]. The alternating prevalence of these two major types, with epidemics of each type with an interval of approximately 8-10 years, may be a reflection of strain competition. A similar if not as clear a pattern of fluctuation of these types has occured also in other Nordic countries. Epidemics by M/*emm*1 were observed in 1988 in Sweden and Norway (synchronously with Finland), during 1999-2002 in Denmark and during 1994-1995 again in Sweden [89, 96, 219, 300, 305]. Similarly, M/*emm*28 was dominant during 1996-1997 in Sweden and during 2003-2004 in both Sweden and Denmark (synchronously with Finland) [98, 200]. However, some caution should be used in comparing these findings given the different typing methods employed. As said before, even though with types 1 and 28, the T, M and *emm* typing results correlate reasonably well, some variation in the strains is evident and uncommon type combinations are encountered [159].

The reasons for the seemingly constant flux in the type prevalence are not yet clear. One suggested explanation is the tendency of a population to develop immunity after a certain time towards a prevalent type, causing the type to decrease in prevalence, creating a niche for other types. In a similar fashion, the *emm* types of pharyngeal paediatric isolates seem to have age-associated differences, which may be a reflection of an aquired immunity towards more common types as a consequence of exposure in early life [151].

Type *emm*28 and more rarely also *emm*4 have been shown to be associated with postpartum infections and puerperal sepsis [51, 59, 323, 322, 333]. Similarly, in this study, *emm*28 infections were concentrated in females of child-bearing age. However, estimations of how many of these infections may have been pregnancy-related were beyond the scope of this study. No particular *emm* types were found to be concentrated specifically in males.

The rapid emergence of an uncommon type, *emm*84, in 2006-2007 was of specific interest because of the low prevalence of this type in other countries. These isolates were found to be mostly of clonal origin. The analysis of medical records of a cluster of *emm*84 cases could not identify a common exposure or contacts between the patients. Instead, it became evident that all patients had one or more

predisposing factors for infection, emphasizing the role of these factors in invasive disease.

During the 1990s, type *emm*84 was found to predominate among erythromycin-resistant strains causing noninvasive infections in children in Greece, and has also been a common type in the UK [88, 336]. Apart from these findings, only sporadic infections by *emm*84 have been reported [181, 201, 260, 307, 314]. However, even though it appears that *emm*84 emerged as a new type in Finland, one cannot be certain that this type has not been present before, as extensive data on *emm* typing exists only from 2004 onwards. Type *emm*84 would not have been detected with T typing, the primary typing method until 2003, as it is nonspecific for this *emm* type. *emm*84 is not included in the putative 26-valent vaccine currently under clinical trial. The vaccine types may need re-evaluation in the future in order to adapt to changes in *emm* type prevalence to ensure the vaccine's coverage [56, 179, 216, 242].

A high proportion of Finnish iGAS isolates were susceptible to the antimicrobial agents tested. The overall erythromycin resistance during 2004-2007 was low, having been in decline during this decade in both blood and pharyngeal isolates [108]. This is regarded to be a consequence of nationwide restrictions on the use of antimicrobials. Finnish *S. pyogenes* strains remain susceptible to clindamycin. Denmark and the USA have reported similar low resistance rates [198, 260]. Tetracycline resistance in Finnish iGAS isolates were found to be associated with certain clones, as has also been found previously, with the resistant strains being mainly uncommon *emm* types [172].

This study also addressed the usability of different typing methods for the purpose of epidemiological surveillance. T serotyping alone was found to be an insufficient method for determining clonality in the Finnish material, with several genotypes existing among the serotype T28 isolates (publication I). Generally, *emm* typing is the method of choice for characterisation of GAS isolates in many countries, including Finland. However, when the Finnish *emm*84 isolates were further characterised with PFGE and SAg profiling, they were found not to be of clonal origin (publication II). For some *emm* types, such as *emm*1, two or more circulating subtypes can be found at the same time and they can be considered epidemiologically as distinct types, but with further characterisation, isolates of a specific subtype may also prove to be nonclonal. Therefore, for the purpose of general epidemiological surveillance, *emm* typing alone is a sufficient and cost-effective method, but for cluster or outbreak investigations, it should be complimented with PFGE typing, and if applicable, with other methods such as SAg profiling or antimicrobial susceptibility testing. When both *emm* and PFGE typing

are performed together, they provide the sufficient discriminatory power needed for clonality analyses. However, the use of PFGE for typing of all isolates is not feasible as it is very time-consuming, the same being true for MLST. The implementation of MLST, instead of PFGE, into the typing scheme to supplement the *emm* typing results would offer some advantages for clonality analyses, but compared to PFGE, MLST lacks discriminatory power.

6.3 Streptococcal non-necrotizing cellulitis

In the case-control study of acute bacterial non-necrotizing cellulitis, strikingly, the most common bacterial finding was of group G streptococcus (*S. dysgalactiae* subsp. *equisimilis*) instead of GAS. Some of the case-patients and their household members also carried GGS as part of their pharyngeal flora, whereas it was not found in control subjects. GGS was isolated either from the skin or blood in 22% of patients, while GAS was isolated only from 7% of patients. This finding was in contrast to earlier knowledge of cellulitis which regarded the group A streptococcus (*S. pyogenes*) as the main causative agent [48, 94, 304]. However, some other studies have noticed a stronger role of GGS in acute cellulitis than expected [94, 148], and a recent case-control study in Iceland found these bacteria in similar proportions as in our study [35]. In our study, the proportion of patients with a positive blood culture result (2%) was in the expected range for this disease [31, 37, 94, 161]. This study found a slight but non-significant male predominance among patients presenting with acute cellulitis, also been found by others [35, 94, 191, 223]. Presumably the sex-specific differences in risk factors play an equally important role in cellulitis as in invasive infections.

Group G streptococci (*S. dysgalactiae* subsp. *equisimilis*) are known to be closely similar to GAS in their genetic and molecular properties, as well as in the clinical spectrum of infections, and similar predisposing factors have been identified for infections by both organisms [134, 160, 163]. Research strongly supports that horizontal transfer of virulence genes occurs between GAS and GGS strains [74, 75, 165]. GGS strains have been increasingly recognised as a cause of pharyngitis, skin and soft tissue infections, bacteraemia and toxic shock [209, 332]. Similarly, studies from Denmark and Israel have noticed an increase in the frequency of GGS bacteraemias during the 1990s, with a probable source of skin or soft tissue infection identified in the majority of cases [134, 306]. Our knowledge of the prevalence of GGS bacteraemia in Finland is limited, since it is not among the notifiable diseases. A population-based study undertaken in one healthcare district in Finland found that the incidence of GGS bacteraemia was higher than GAS bacteraemia and had a similarly increasing trend [256]. Seventy percent of the bacteraemic GGS infections (equally many as with GAS bacteraemias) presented with a skin or soft tissue

infection. A change in the epidemiology of β-haemolytic bacteraemia may be occurring, and possibly also with cellulitis towards an increasing aetiological role of GGS over GAS [306].

The finding that only 13% of the cellulitis patients were carrying β-haemolytic streptococci in their throat is concordant with other studies [94]. GGS was more commonly recovered from the throat than GAS, similar to an Australian study on the epidemiology of GGS [209]. It has been assumed that throat carriage of GAS and group C or G streptococci may be independent of each other and almost mutually exclusive [209]. This study was in line with this hypothesis. One of the patients in this study harboured the same GGS strain in both the pharynx and affected skin. In this case, it is impossible to say if the respiratory tract is the reservoir for the pathogen. However, colonisation of skin prior to infection has been reported and it is a far more likely origin to the infection [161].

In this study, there was no predominance of a specific *emm* type of GGS or GAS associated with the disease. The knowledge of *emm* types of GGS and GAS causing cellulitis infections is very limited. In an earlier study in Sweden, serotypes T1 and T8 dominated in GAS isolates from cellulitis patients [240]. Many of the GGS *emm* types found in this study have been mentioned in relation to invasive disease [57, 127, 165, 196]. Similarly, the *emm* types of GAS found in cases of cellulitis (including *emm*28) were also encountered among the invasive isolates of this study. The small number of GAS isolates found in cellulitis infections did not allow for detailed comparisons of the *emm* types with the invasive isolates.

A limitation to this study is that the case patient population consisted of hospitalised patients with cellulitis infections of intermediate severity. The proportion of patients treated on an outpatient basis is not known. The Finnish treatment recommendation is that febrile patients with cellulitis should be hospitalised for initial parenteral antimicrobial treatment. The most severe cases of cellulitis, e.g. patients requiring intensive care treatment or surgery, were not eligible for inclusion in this study as they were treated in other wards. This fact may have lowered the observed rate of bacteraemia and the rate of recurrence, which may be further underestimated by the short study period and lack of follow-up [62]. As such, the true incidence of these infections in Finland cannot be estimated based on this study. Comparisons between countries are also complicated by the broad spectrum of severity of these diseases and differences in treatment practices. A study from the Netherlands stated that only 7% of all patients presenting with cellulitis of the leg were hospitalised, and another study from the USA estimated that 5.7% of cellulitis patients were treated in in-patient hospital settings (such as our study) and 20.5% in acute care settings and outpatient hospitals [91, 116]. In another approximation from the UK, of those with

three or more episodes of cellulitis, half of recurrences led to hospital admission [62].

As a further limitation to this study, the possibility cannot be excluded that the choice of sampling method and, for some cases, antimicrobial treatment prior to sampling (in 28% of episodes) may have affected the bacterial findings. By using a non-invasive sampling method, β-haemolytic streptococci could be isolated from one-third of the samples taken. However, when the infection site was intact, the sample was taken from the suspected site of entry, such as any abrasion or fissured toe-web, if such a site was detected. The findings differed by sampling site, and more than half of the isolates (and more specifically, GGS) were obtained from the suspected site of entry, which may or may not have been the actual site of entry of the pathogen. Nevertheless, recent findings support the role of toe webs as a potential site of entry, with colonisation of toe webs by pathogens having been identified as a risk factor for lower-limb cellulitis [35, 133].

One further limitation concerns the size and duration of the case-control study. Even though a study including almost a hundred case patients and control subjects within a one-year period was considered to be large enough to enable conclusions with sufficient statistical power, a study with longer duration and a follow-up period would have been of benefit to obtain more microbiological isolates and considerably more information about the recurrences and seasonal trends of infections. Inclusion of twice the number of controls per case patient would have helped increase the power of the study.

6.4 Recurrent cellulitis

Within the one year study period, six (7%) of the patients had a recurrence of cellulitis, with four patients having two disease episodes and two patients having three episodes. According to earlier studies, in 20-30% of patients with bacterial cellulitis the infection recurs within a three-year follow-up period [94, 162]. GGS could be isolated in three patients and GBS in one patient of the six patients with recurrences. For two of these patients, the consecutive disease episodes could be confirmed as being caused by an identical clone of GGS, strongly supporting the concept of a relapse. Whether recurrences of cellulitis are specifically associated with a streptococcal species (namely, *S. dysgalactiae* subsp. *equisimilis*), or even a specific streptococcal strain, is yet undefined. GGS has been known to cause recurrent bacteraemia [57]. The notion of the same streptococcal strain causing a relapse raises the question of how the pathogen evades the antimicrobial treatment. Intracellular persistence during acute soft tissue infections may be a mechanism of GAS for avoiding eradication by the antimicrobial [317]. The recurrences may also

be associated with individual variation in immunological response, with some patients more susceptible to the infection and unable to clear the bacteria from the infection site.

As many as 50% of the case-patients in this study reported having suffered previous episodes of cellulitis prior to this study. In addition to various general and local risk factors for infection, such as oedema, disruption of the cutaneous barrier, and being overweight, a previous cellulitis infection in itself seems to predispose to future episodes by causing scarring and damage to tissue and lymphatic circulation [9, 35, 62, 94, 191, 223, 265]. The median age of the case-patients with recurrences was 48 years; 10 years less than that of other case-patients. All case-patients were also studied for risk factors for infection (outside the scope of this PhD thesis), and although the study was underpowered to identify risk factors specifically for recurrences, oedema, broken skin, and being overweight were identified as risk factors for an acute cellulitis infection [169]. In one retrospective study from France, age (and to a lesser extent, sex) was identified as a relevant factor for recurrent infections, with younger patients having a higher risk for recurrences [191].

Streptococcal cellulitis remains a disease of high morbidity [92]. Recurrences represent a major problem affecting patient's quality of life and incurring considerable costs in the form of hospitalisation, sick leave, and disability. It has been estimated that a large proportion of these infections could be preventable by increased awareness and management of toe-web intertrigo and by adequate treatment of dermatomycosis (fungal infection), a key risk factor for infection [35, 265].

6.5 Seasonality of *S. pyogenes* infections

Seasonal variation in iGAS disease incidence in Finland was observed, with occasional peaks of cases occurring during midwinter and midsummer. In Europe, the USA, and Canada, peaks are generally observed during the first months of the year (winter and spring) [72, 185, 244, 322, 334]. The unusual pattern and irregularity of the seasonality observed in Finland may be partly due to natural variation given the reasonably small study size. Several factors may be associated with seasonality, including environmental changes (temperature, humidity, annual light/dark cycle) or host-behavioural patterns (differences in outdoor activities, depending on sex and academic season) [82]. Viral respiratory infections may predispose to bacterial infections, as has been shown to occur with pneumococcal infections [175, 308]. Presumably there are different predisposing factors and underlying conditions associated with the two distinct peaks. In a study within one healthcare district in Finland, the predisposing factor in over half of GAS

bacteraemias was disruption of the cutaneous barrier, which may occur predominantly in the summer season [256].

In the case-control study, more episodes of acute cellulitis occurred during the summer (July-September) than in other study periods. A study from the USA also concluded that cellulitis was most common during the summer and least common during the winter [91]. A hospital in the UK reported seeing a marked seasonal variation in cellulitis with a peak of cases in May [129]. A case-control study reported more cases of cellulitis during the summer in Tunis [223], where the climatic conditions during the hot season nonetheless differ considerably from those of Finland. In contrast, some studies have observed more cellulitis cases in the winter or no seasonal variation at all [48, 94, 148, 161]. Nevertheless, skin infections in general may be more common during the summer, due to increased outdoor activities, swimming, and increased exposure of the skin to insect bites and minor trauma, for example during gardening work [129, 264]. Furthermore, the higher temperature and humidity in the summer may delay the healing of wounds and increase predisposition to toe-web intertrigo, providing a portal of entry for the pathogen and increasing the risk of cellulitis.

6.6 Outcome of *S. pyogenes* infections

The overall case fatality of invasive GAS infections in Finland was 8% during 2004-2007, which is low compared to rates encountered in some European countries and the USA [72, 90, 184, 244]. A sudden peak in the case fatality (12%) occurred in 2005, partly explained by the changing *emm* type distribution. The proportion of infections by types associated with a higher than average case fatality, namely, *emm*1 and *emm*75, increased. In parallel, the proportion of *emm*28 decreased, this type being associated with a lower than average case fatality. The case fatality of infections by *emm*84 was of specific interest because of its rareness and rapid emergence in Finland. The overall CFR associated with *emm*84 infection was 7%, not differing significantly from the average case fatality. Sixty per cent of the fatal cases were associated with the five most common *emm* types, thus, the case fatality was affected mostly by changes in their prevalence.

Although infections by specific *emm* types were associated with different rates of case fatality, the virulence of a given strain is not solely due to the properties of the M protein, but rather a combination of the strain's various virulence factors. Even though certain SAg profiles have been found to be more frequent among invasive isolates, the association between pathogenic potential and the presence of single SAg genes is not completely clear [49, 72]. Furthermore, properties of the host, such

as age, underlying diseases, and the function of the immune system are vital in determining the course and outcome of infection [15, 292].

Comparisons of case fatality rates between different studies are complicated by differences in case definitions, such as the inclusion or exclusion of nonbacteraemic cases with other sterile site isolations and/or STSS, the timing of death (7-day, 30-day or in-hospital mortality), and sources of outcome information (e.g. population registry vs. hospital records). In this study, 7-day mortality information was obtained from the Population Information System instead of hospital records. A strength of the outcome analyses is the completeness and coverage of the registry-based information. However, the same limitation of the case definition affects the case fatality rates. The exclusion of non-bacteraemic cases with other sterile site isolations such as joint aspirates, or cases of STSS without bacteraemia, may have an increasing or decreasing impact on the observed case fatality rate.

Although cellulitis may be fatal, usually these cases are associated with low mortality. The severity of illness has been shown to increase with age but also with the presence of comorbidities such as diabetes [116]. Patients who produce higher levels of cytokines in response to streptococcal SAgs have been shown to develop more severe systemic manifestations [236]. The intracellular presence of the pathogen, which has been shown to occur with acute soft tissue infections, may lead to attenuated immune response, characterised by the lower level of inflammation [317]. Thus, the reason for low mortality due to cellulitis may be more related to the properties of the human immune response than of the pathogen. Patients with cellulitis may also produce higher levels of toxin-neutralizing antibodies than patients with sepsis [240].

7 CONCLUSIONS AND FUTURE CONSIDERATIONS

The incidence of invasive group A streptococcal (*S. pyogenes*) disease has had an increasing trend during the last ten years in Finland, following a general European pattern and remaining at a relatively high level. In general, small children and the elderly had the highest incidence, although the rates in these age groups in Finland have remained at a similar or lower level than in other Nordic countries. Presumably differences in the predisposing factors and underlying conditions contribute to the observed age- and sex-specific differences in the incidence rate. Collection of clinical data is needed in order to fully understand the influence of these factors and to try and prevent infections in specific risk groups. Setting-up a nationwide collection of clinical data would demand considerable personnel resources in the healthcare districts, and for this reason such a study has recently been performed only within a defined area of Pirkanmaa district. Future studies could be targeted at the largest healthcare district, the Helsinki metropolitan area, in order to ensure that the study has enough power to detect possible associations, for example between disease manifestations and certain *emm* types.

The incidence of iGAS disease in Finland is influenced by dynamic and rapid changes occurring in the strain type distribution due to inter-strain competition. Two strain types, T/*emm*1 and T/*emm*28, were common throughout the study period and their prevalence fluctuated in turn with each other. The prevalence of infections by type *emm*1 had the highest impact on incidence, but the sudden emergence of *emm*84 and other less common types also contributed to the increase. The *emm* types of the putative recombinant M-protein based vaccine would have covered only half of the isolates during recent years due to unique features in the Finnish type distribution as compared to that in other developed countries.

A new and unexpected finding of this study was that instead of group A streptococcus, group G streptococcus (*S. dysgalactiae* subsp. *equisimilis*) predominated in acute cellulitis infections in Finland. A predominance of GGS was also seen in the pharynx of case-patients and their family members, whereas the control subjects did not harbour GGS. No specific *emm* type of GGS or GAS associated with the disease. The recurrent nature of cellulitis became evident during the study and in two recurrences, the consecutive disease episodes were caused by an identical clone of GGS.

The molecular epidemiology of group G streptococcus, being genetically close to GAS and causing clinically similar infections, would be worthy of further research. Sequencing of the streptococcal genomes of other species than *S. pyogenes* would bring more perspective to the research of streptococcal diseases [142]. Also, further

studies of the inflammatory responses during a cellulitis infection would shed more light on why these infections tend to persist, and possibly provide a means to prevent recurrences, decreasing the suffering of patients and hospitalisation costs. The GGS strains found in this study could be further characterised to examine their virulence properties, for example with SAg profiling.

A seasonal pattern with occasional peaks of cases during the midwinter and midsummer was observed in invasive infections, differing from the general seasonal trend of a peak in the winter or spring. Presumably these peak seasons are associated with different predisposing factors and underlying conditions. Comparisons of seasonal patterns between neighbouring countries and on different hemispheres could yield more information on the undoubtedly multiple factors associated with seasonality. More cellulitis infections were also found to occur during the summer, which may be associated with predisposition to toe-web intertrigo due to higher temperature and humidity during the summer.

The case fatality of *S. pyogenes* infections was found to be at a reasonably low level in Finland compared to other countries, despite a temporary peak in the case fatality during the latest study years. Cases with type *emm*1 were associated with higher than average case fatality, whereas *emm*28 infections were associated with lower than average case fatality. Dynamic changes in the strain type prevalence had an influence on the incidence and case fatality, with changes in the prevalence of the most common types such as *emm*1, *emm*28 and *emm*84 having the highest impact. These strains could be further characterised for their SAg profiles and other virulence factors in relation to outcome.

The *emm* typing method was found to be sufficient for the purpose of general epidemiological surveillance; higher discriminatory power could be achieved when complementing it with PFGE. Research into the molecular epidemiology of streptococci would benefit from development and implementation of novel molecular methods for typing and characterisation. Ideally, a typing method would provide a "fingerprint" of selected characteristics of the strain (e.g. virulence, genetic origin, antimicrobial resistance), have good discriminatory power for detecting clonality, and be quick and easy to perform. The oligonucleotide microarray technology could offer a versatile solution for the characterisation of GAS.

This study adds to our understanding of the molecular epidemiology of *S. pyogenes* infections in Finland and provides a basis for comparison to other countries and future trends. Global *emm* type surveillance and outcome analyses remain important in order to rapidly detect changes in the type distribution that might lead to increase

in the incidence and mortality. Early identification of iGAS infections is of importance in the initiation of prompt treatment to improve patient outcome. Monitoring of antimicrobial resistance is needed in order to react to a possible resistance problem in a timely manner with appropriate national guidance on choice of antimicrobials. Identification of strain types serves as basis for studies of disease pathogenesis and vaccine development.

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